University of Sussex

Foraging ecology and conservation of honeybees, bumble bees and solitary bees

Georgia Hennessy

Submitted for the degree of Doctor of Philosophy

Submission date: April 2021

University of Sussex

Declaration

I declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research. Any help provided by individuals with data collection and analysis is fully acknowledged.

I certify that this thesis has not and will not be submitted- in whole or part, to another university for the award of any other degree

Signature

Georgia Hennessy

General summary

This thesis contributes to two inter-related fields of research: bee conservation and bee foraging ecology. The first focuses on solitary bee ecology, identifying forage and habitat requirements along with educating the general public on aspects of solitary bee behaviour to aid in the conservation of the studied species. These results greatly improve our understanding of two rare and understudied species of solitary bee in the UK, *Eucera longicornis,* the long-horned bee, and *Anthophora retusa*, the flower potter bee, along with information on the forage requirements of a common and non-native bee species, *Colletes hederae,* the ivy bee, confirming its specialisation and reliance on the plant species *Hedera helix,* common ivy. The information provided by this research on the two rare species is currently being used by stakeholders to help conserve populations found on their land. The second focuses on how an important but understudied environmental factor, wind, influences bee foraging behaviour. Two types of common social bees, honey bees and bumblebees, that are major pollinators were studied foraging on both artificial and natural flowers, with implications for increasing our understanding of the potential future impacts of climate change on bees. Among other things, the wind research has identified a novel part of foraging behaviour much influenced by wind, hesitancy to take-off from flowers, which increases at higher wind speeds, and results in significant decreases in flower visitation rate. This thesis has contributed novel knowledge to our understanding of the foraging ecology of two rare bee species in the UK and has identified that having a wide foraging breadth does not necessarily mean a species will be common, as was found to the case for *A. retsua.* Foraging behaviour was also found to be influenced by the understudied environmental variable, wind, with it being found to reduce the foraging efficiency of honey bees and increase both honey bees and bumble bees hesitancy to take off.

Chapter Two studied the population of the rare solitary bee, *Anthophora retusa*, living at Seaford Head in Sussex, one of the 5 sites it is known from in Britain. Results showed that it forages on a range of flower species, including the very common *Glechoma hederacea* (ground ivy). The population is small (male population size in 2019 was estimated to be around 160 individuals). Transect surveys showed that the species is restricted to a very small area, c. 30ha area within the Seaford Head reserve.

Chapter Three shows that the two populations of the rare *Eucera longicornis* found on Gatwick Airport land had estimated populations of 300 females and 130 males in 2019, and that these populations remained approximately stable over the three years of data collection. *Eucera longicornis* females collected pollen predominantly from species within the Fabaceae family, which were highly abundant within the 500m surrounding the aggregations and hence are likely to be key to their success.

Chapter Four confirmed that ivy, *Hedera helix* is the predominant floral resource for the solitary bee, *Colletes hederae* (ivy bee) in Sussex, with ivy comprising 98% of pollen samples collected from females. Female *C. hederae* activity was synchronised with ivy bloom. However, *C. hederae* females did collect pollen from other plant species before ivy was in peak bloom. *C. hederae* was the most abundant species foraging on ivy, even when honeybee hives were present in the local area.

Chapter Five used artificial flowers and wind generated by fans and found that increasing wind speed caused a significant reduction (37%) in flower visits for foraging *Apis mellifera*. This reduction was due to an increase in 'hesitancy', the time to take off from a flower once a bee had finished probing. The indirect effect of flower movement had no effect on flower visitation rate. However, it did cause an increase in flight duration but this was offset by a decrease in search time once a bee was on a flower.

Chapter Six found that when foraging on natural flowers of lavender and marjoram *Apis mellifera* flower visit rate decreased with increasing wind speed due to an increase in handling time per flower. The influence of wind speed on flower visit rate differed between lavender and marjoram, with a sharper reduction when foraging on marjoram. This was not explained by differences in flower movement speed, with flower movement not influencing flower visit rate.

Chapter Seven found that when foraging on lavender, *Apis mellifera* flower visit rate decreased with wind speed, whereas *Bombus* species flower visit rate was unaffected. However, both species did experience an increase in handling time per flower with increasing wind speed. Also, the number of *Bombus* foragers on a patch with wind was significantly lower than on a patch where no wind was present, indicating when given the option they will choose to forage in lower wind speeds.

Chapter Eight identified that hesitancy to take off increased with wind speed for both *Apis mellifera* and *Bombus* species when foraging on seven plant species in naturally varying wind

conditions. Hesitancy duration in relation to wind speed did not differ between plant species. However, independently of wind speed it did. *Bombus* hesitancy was found to increase significantly more with increasing flower movement when compared to *A. mellifera.*

Publications arising from this thesis

Hennessy G., Harris C., Pirot L., Lefter A., Goulson D., Ratnieks F.L.W. Wind slows play: increasing wind speed reduces flower visiting rate in honeybees. *Animal behaviour* (accepted) (**Chapter Six**)

Author contribution to chapter six: CH, LP, GH, and AL were involved in data collection. GH analysed the data and wrote the final manuscript. FR and DG assisted with experimental design and editing of the manuscript.

Hennessy, G., Uthoff, C., Abbas, S., Quaradeghini, S. C., Stokes, E., Goulson, D., & Ratnieks, F. L. (2021) Phenology of the specialist bee *Colletes hederae* and its dependence on *Hedera helix* L. in comparison to a generalist, *Apis mellifera*. *Arthropod-Plant Interactions*, 1-13. (**Chapter Four**)

Author contribution to chapter four: GH, SA, SQ, ES and CR all contributed to data collection. GH analysed the data and wrote the manuscript. DG and FR proof read and made edits to the manuscript with FR devising the initial idea

Hennessy G., Goulson D., Ratnieks F.L.W. (2020) Population assessment and foraging ecology of the rare solitary bee *Anthophora retusa* at Seaford Head Nature reserve *Journal of Insect Conservation* Vol:1-15 (**Chapter Two)**

Author contributions to chapter two: GH conducted data collection and completed data analysis and wrote the manuscript. DG and FR proof read and edited the manuscript and assisted in method design.

Hennessy G., Goulson D., Ratnieks F.L.W. (2020) Population assessment and foraging ecology of nest aggregations of the rare solitary bee, *Eucera longicornis* at Gatwick Airport, and implications for their management *Journal of Insect Conservation*, *24*, 947-960 (**Chapter Three)**

Author contributions to chapter three: GH conducted data collection and completed data analysis and wrote the manuscript. DG and FR proof read and edited the manuscript and assisted in method design.

Hennessy, G., Harris, C., Eaton, C., Wright, P., Jackson, E., Goulson, D., & Ratnieks, F. F. (2020). Gone with the wind: effects of wind on honey bee visit rate and foraging behaviour. *Animal Behaviour, 161*, 23-31. (**Chapter Five**)

Author contributions to chapter five: GH, CH, CE, PW, EJ all contributed to data collection. GH analysed the data and wrote the manuscript. DG and FR proof read and edited the manuscript and assisted in designing the experiment.

Hennessy, G., Balfour, N. J., Shackleton, K., Goulson, D., & Ratnieks, F. L. (2020). Stinging risk and sting pain of the ivy bee, *Colletes hederae*. *Journal of Apicultural Research*, *59*, 223-231. (**Appendix A**)

Author contributions to Appendix A: GH, KS, NJ and FR all contributed to data collection. GH analysed the data and wrote the manuscript. DG and FR proof read and edited the manuscript and assisted in designing the experiment.

Papers currently in submission

Hennessy G., Harris C., Pirot L., Lefter A., Goulson D., Ratnieks F.L.W., (*in submission at Apidologie*) Blowin' in the wind: Honeybee but not bumblebee flower visiting rate is significantly reduced with increasing wind when foraging on lavender (*Lavandula* spp) **(Chapter Seven)**

Author contribution to chapter seven: CH, LP, GH, and AL were involved in data collection. GH analysed the data and wrote the final manuscript. FR and DG assisted with experimental design and editing of the manuscript.

Hennessy G., Rantnieks F.L.W, Goulson D., (*in submission Journal of Apicultural Research*) Wind beneath my wings: Increasing wind speed causes increased hesitancy to take-off in foraging honey bees and bumblebees **(Chapter Eight)**

Author contributions to chapter eight: GH conducted data collection and completed data analysis and wrote the manuscript. DG and FR proof read and edited the manuscript and assisted in experimental design.

Acknowledgements

Firstly, I would like to thank both my supervisors, Francis Ratnieks and Dave Goulson. I know I wasn't their first choice, but I hope I have managed to surpass their initial expectations. Without their vast combined knowledge, patience and ability to read endless papers on wind I would not have managed to make it to the end.

I am of course grateful to Gatwick Airport and the Sussex Doctoral school for funding this PhD. Also thank you to Gatwick for trusting me enough to allow me to stand in a high visibility jacket, waving my insect net like a maniac on their land in the name of research. Thank you to Rachel Bicker, for all her help both with data collection and figuring out the logistics of working on airport property.

I would also like to thank everyone in the Laboratory of Apiculture and Social Insects, without whose constant support, both through advice and biscuit giving, I would not have made it through this PhD. Thank you to Nick Balfour, Luciano Scandian, Kyle Shackleton, Ciaran Harris and Karin Alton. Also, a special thanks to Veronica Wignall and Owen Middleton, who both had to put up with panicked conservations about statistics more times than anyone should have to.

Thank you to Maria Cala Castellamos and Alan Stewart for your guidance and advice throughout my PhD.

A special thanks to everyone who helped with data collection, especially during the colder months when the last thing anyone wants is to be stood in a shed and blasted with a fan. Thanks to Charlotte Eaton, Paul Wright, Emily Jackson, Cassanda Uthoff, Sema Abbas, Stefano Quaradeghini, Emma Stokes, Lucien Pirot, Alex Lefter, Natalie Cook and Rosalind Henderson.

A huge thank you to my friends and family who helped me throughout my four years. My mum and dad, Vanessa and Karl Hennessy, who were always my biggest fans. Thank you to Mark and Simon for their wittering's, and all the other podcasts keeping me sane during those days of solo data collection, and hello to Jason Isaacs. Of course, a huge thank you to Toby Collingwood, who was always willing to provide an outside opinion and sometimes even a shoulder to cry on. As summed up by Charles Darwin, on some days *"I hate myself, I hate clover and I hate bees"*.

They say it takes a village to raise a baby, I would say the same about completing a PhD.

Table of Contents

Declaration ..... **i**

General summary ..... **ii**

Publications arising from this thesis ..... **iv**

Acknowledgements ..... **vi**

Chapter One: General Introduction **1**

Chapter Two: Population assessment and foraging ecology of the rare solitary bee *Anthophora retusa* at Seaford Head Nature reserve. **12**

2.1 Abstract 12

2.2 Introduction 13

2.3 Methods 16

2.4 Results 22

2.5 Discussion 32

Chapter Three: Population assessment and foraging ecology of nest aggregations of the rare solitary bee, *Eucera longicornis* at Gatwick Airport, and implications for their management. **37**

3.1 Abstract 37

3.2 Introduction 38

3.3 Methods 40

3.4 Results 45

3.5 Discussion 51

Chapter Four: Phenology of the specialist bee *Colletes hederae* and its dependence on *Hedera helix* L. in comparison to a generalist, *Apis mellifera.* **59**

4.1 Abstract 59

4.2 Introduction 60

4.3 Methods 62

4.4 Results 68

4.5 Discussion 76

Chapter Five: Gone with the wind: Effects of wind on honey bee visit rate and foraging behaviour **82**

5.1 Abstract 82

5.2 Introduction 83

5.3 Materials and methods 84

5.4 Results 89

5.5 Discussion 95

Chapter Six: Wind slows play: Increasing wind speed reduces flower visiting rate in honeybees **99**

6.1 Abstract 99

6.2 Introduction 100

6.3 Methods 101

6.4 Results 105

6.5 Discussion 110

Chapter Seven: Blowin' in the wind: Honeybee but not bumblebee flower visiting rate is significantly reduced with increasing wind when foraging on lavender (*Lavandula spp.*) **115**

7.1 Abstract.....115
7.2 Introduction.....116
7.3 Methods.....117
7.4 Results.....120
7.5 Discussion.....124
Chapter Eight: Wind beneath my wings: Increasing wind speed causes increased hesitancy to take-off in foraging honeybees and bumblebees.....128
8.1 Abstract.....128
8.2 Introduction.....129
8.3 Methods.....130
8.4 Results.....134
8.5 Discussion.....138
Chapter Nine: General Discussion.....142
References.....150
Appendix A- Stinging risk and sting pain of the Ivy bee, Colletes hederae.....181
Appendix B- Chapter two.....202
Appendix C- Chapter three.....208
Appendix D: Chapter Seven.....216
Appendix E: Chapter Eight.....217

Chapter One: General Introduction

1.1 Bee and pollinator biodiversity

Globally there are an estimated 20,000 species of bee (López-Uribe et al 2017). Insect pollinators, including bees, are essential not only for the pollination of approximately 70% of angiosperm species (Schoonhoven et al 2005), but also of many crops. Approximately 85% of European (Williams 1994) and 70% of the Earth's (Klein et al 2007) crop species benefit from pollination by insects. Although other pollinator groups, such as hoverflies (Syrphidae) and beetles (Coleoptera), do contribute, bees (Apoidea) are considered to provide the majority of these crop pollination services (Williams 1994, Klein et al 2007). However, as discussed by Senapathi et al. (2015a), the ethical, economic, and biological reasons for conserving insect diversity extend beyond those species that contribute to cop pollination, although these are often not the focus of conservation efforts (Senapathi et al 2015a). For example, insect species which may not contribute to the pollination of crops may pollinate wildflower species which provide food and shelter for other wildlife, including beneficial invertebrates that reduce crop pest abundance (Ditner et al 2013), along with species of birds, bats and rodents.

Although the rate of global insect decline is currently under debate (Thomas et al 2019), there is no doubt that many insect species are in decline (Biesmeijer et al 2006, Potts et al 2010, Didham et al 2020). For example, 55% of common butterfly species in the Netherlands have suffered declines in both abundance and distribution between 1992 and 2007 (Van Dyck et al 2009), and in Germany a study examining flying insect biomass in protected areas found a decline of 76% over 27 years (Hallmann et al 2017). Globally, 25% of bee species have not been reported between 2006 and 2015 (Zattara and Aizen 2021). Although globally there is an average decline of pollinator diversity and abundance (Potts et al 2010) this trend is not necessarily true for all pollinator species at some smaller scales. For example, the 2019 results of the UK Butterfly Monitoring Scheme indicated it was the best year for butterfly species for 20 years, with four species (Chequered Skipper, Orange-tip, Brimstone, and Marbled White) having their best year on record. Just over half of UK Butterfly species showed higher population levels compared to 2018. However, even with the positive increase in many of the UK species, since the 1970s more species have been declining in abundance than increasing (JNCC, 2020).

In the UK since 1880, 23 bee and flower-visiting wasp species have gone extinct (Ollerton et al 2014). However, three new bee species have also recently arrived in Britain. One bumblebee species, *Bombus hypnorum,* and two non-eusocial (solitary) species, *Colletes hederae* and *Hoplitis adunca.* The latter is restricted to a small area in London (BWARS 2016), but the other two are now found over much of Britain (Crowther 2017, BWARS 2017).

Insects face many challenges, with the potential to act singly, additively or synergistically to contribute to declines (Potts et al 2010). Pesticides (Brittain et al 2010, Goulson et al 2015), disease (Goulson et al 2015), habitat loss (Potts et al 2010), and climate change (Forrest and Chisholm 2016, Soroye et al 2020) have all been identified as significant. Pesticides are known to negatively influence both honeybees and wild bees in a variety of ways. For example, honeybee colonies in Hungary that had foraged on crops treated with clothianidin had lower over winter worker survival (Woodcock et al 2017), and wild bee density has been found to be lower in areas surrounding crops treated with neonicotinoids vs untreated crops (Rundlöf et al 2015). Exposure to pesticides increases the risk of disease for bee species due to their immunosuppression effects (Sánchez-Bayo et al 2016).

Habitat loss is also a long-term contributor to bee declines (Goulson et al 2008, Brown and Paxton 2009, Potts et al 2010). The intensification of farming and changes in farming practices since the 1920s has resulted in major losses of semi-natural flower rich habitats (Ollerton et al 2014). In the UK, 97% of flower rich grasslands were lost in the 20th Century (Howard et al 2003), which has resulted in range contractions of bee species associated with this habitat, for example long tongued bumblebees (Goulson et al 2008). Urbanisation also poses a risk for bee species. Despite urban areas covering a small portion of the globe (~ 2%, Svirejeva-Hopkins et al 2004) they have had detrimental effects on some insect species. For example, in a study examining bees and wasps in Belo Horizonte, Brazil, the abundance of stingless bees was negatively affected by the increase of buildings and loss of vegetation cover associated with urbanisation (Zanette et al 2005). Although urbanisation and land use change does negatively affect many bee species, appropriate management of previous industrial sites can be used to help species conservation. For example, in the UK, 194 invertebrate species of conservation importance have been recorded from brownfield sites, with 17 of these being priority species within the UK Biodiversity Action Plan (Bodsworth et al 2005, Macadam and Bairner 2012). Managed powerline strips in parts of the US were found to contain twice as many species compared to the adjacent either oak pine or evergreen

forest plots and contained two rare species of solitary bee (Wagner et al 2019). Some urban areas in the UK were found to have higher bee species richness compared to farmland (Baldock et al 2015) and there was higher reproductive output in bumblebee colonies in urban areas than those surrounding agricultural land (Samuelson et al 2018). If managed correctly, human modified environments can act as a refuge for some bee and other pollinator species (Hunter 2014, Baldock et al 2015, Phillips et al 2020, Tew et al 2021).

Another emerging challenge for insect species is climate change. Shifting climates can disturb the synchrony between pollinator and plant emergence (Wilmer 2012, Kharouba et al 2018, Inouye 2020) with this disruption even more of an issue for species which rely on a limited range of forage (Roberts et al 2011). Climate change can also result in existing ranges no longer being suitable due to factors such as warming (Giannini et al 2012). This is being seen with many bumblebee species in North America and Europe, with the southern edges of species' ranges contracting further north in response to increasing temperatures, but the northern edges failing to expand northwards (Sirois-Delisle and Kerr 2018). This results in the shrinking of overall distributions (Kerr et al 2015). Not only is this gradual change in climate impacting pollinator species, but also sudden extreme events. For example, in Florida in January 2010 there was an extreme cold spell, with temperatures reaching record lows for the region (1.6˚C). For four years following this extreme drop in temperature, the abundance of an introduced tropical bee originating from Mexico, *Centris nitida,* was significantly reduced (Downing et al 2016), with this reduction thought to be correlated with the extreme cold event. Extreme storm events, which have been increasing in frequency and severity due to changes in climate (Mirza 2003) can also pose a threat to bee species (Goulson and Nicholls 2016). For example, high levels of rainfall can cause flooding of rivers, potentially destroying the nests of bees in the ground alongside river banks, killing any maturing bees contained within (Fellendorf et al 2004).

1.3 Forage requirements for bees

Bees require flowers for a source of both nectar and pollen, which are their main or even exclusive food and are also used to feed the larvae or provision larval cells. Nectar is the main source of carbohydrates for bees, whereas pollen provides proteins, lipids and other micronutrients (Vaudo et al 2015). When making foraging decisions bees will consider a wide range of information. For example, nectar volume and concentration (Cnaani et al 2006 and Pyke 2016), floral abundance (Fowler et al 2016), pollen quality (Ruedenauer et al 2016),

and flower morphology (Harder 1985). When foraging, bees are not haphazard but integrate these information sources to make non-random flower choices (Pyke 1978).

Some bee species, such as the honeybee, *Apis mellifera,* are generalists and collect nectar and pollen from many species, although each individual forager is usually flower constant on each foraging trip, foraging on only one species of flower (Free 1963, Cane and Snipes 2006). Some species take this specialisation even further and will only ever forage on a single, or a narrow group, of plant species. Bee foraging specialisation is generally classified into three categories; (a) Monolectic, only collecting pollen from a single plant species (b) oligolectic, collecting pollen from a few floral hosts and (c) polylectic, where pollen is collected from a wide range of plants (Cane and Snipes 2006). Although these classifications are constantly being debated, with the idea of monolecty now often replaced with broad or narrow oligolecticy (Cane 2020), they do help describe the different forage requirements of bee species.

Species which are classed as oligolectic, only foraging on a narrow range of plant species normally from within the same family or genus, are thought to be at higher risk of decline or even extinction (Biesmeijer et al 2006, Hofmann et al 2019). For specialist species, habitat change is a major driver for population declines. For example, generalist species tend to be more abundant on farmland due to their more flexible forage requirements (Wood et al 2016). Ground nesting bees which specialised on pollen from the *Larrea* family were lower in abundance compared to cavity nesting or ground nesting generalists in small desert fragments (Cane et al 2006). This was most likely because the availability of their floral resource, *Larrea,* also decreased, meaning small fragments could only support a lower threshold of *Larrea* specialists.

Although agri-environment scheme wildflower seed mixes are becoming more common (Powney et al 2016), a general lack of knowledge regarding the forage requirements of many bee species makes the creating of effective mixes difficult (Wood et al 2017). To determine the forage requirements and therefore specialisation of a bee species requires the use of multiple methodologies. Specialist bees will often visit different plant species for when they collect pollen or nectar. Often, even oligolectic species will visit a broader range of flowers for nectar than for pollen (Minckley and Roulston 2006). Historically, pollen requirements have been determined from pollen masses collected from brood cells (Cane and Snipes 2006). However, this does not give an overall picture of the foraging activities of adult bees and

their nectar requirements (Ritchie et al 2016). The best way to determine the full spectrum of flower usage for a species is likely to be combined analysis of pollen taken from foraging adult females, pollen collection from brood cells and floral visitation data from both sexes (Cane and Snipes 2006). A combination of methodologies not only results in more comprehensive information on forage requirements, but also other foraging behaviours that can only be identified from observing foraging behaviour on flower patches, for example, foraging locations and the dynamics of inter-specific exploitative competition (Chapter Two, Balfour et al 2015, Wignall et al 2019).

By understanding the habitat requirements of species, such as appropriate forage, effective conservation schemes can then be put in place. For example, the introduction of agri-environmental schemes which promote the planting of wildflower strips designed to attract bumblebees are thought to be partially responsible for the increase in some *Bombus* species' distribution (Powney et al 2016).

1.4 Solitary bees

In the UK there are 270 species of bee, with 26 bumblebee species, one honeybee species and the remaining are mainly non-eusocial bees. For example, some species from the family Halictidae are classified as primitively eusocial (Danforth et al 2019), with the extent of social nesting in some of these species varying between high and low elevated populations (Danforth et al 2019). Unlike the eusocial honeybee and bumblebee species, species which are classed as solitary do not live in large colonies. They often have specific nesting requirements, ranging from sloped banks to hollowed out plant stems. Normally, a single female will provision her nest with pollen for the developing larvae. They often have short flight seasons, usually only a few months (Batra 1984), with the adults dying at the end of this time. Offspring will develop and remain in the nest and emerge the following flight season as adults. The time of year when adults emerge from the nest (e.g. early spring or late summer) will determine the speed of development. For example, *Osmia bicornis* (the red mason bee). With this species, the development of egg to adult occurs during the summer, with adults overwintering in a cocoon inside the nest, with emergence in early Spring (around April, Wasielewski et al 2013). In comparison, species which emerge in summer or later generally overwinter as prepupae and develop into adults in the nest in Spring, ready for the summer emergence (Danforth et al 2019).

Although each female solitary bee will build and provision her own nest, often multiple females will create these nests near one another in aggregations, and in some species multiple females will share nest entrances (Batra 1984). With ground nesting species, these aggregations can be particularly dense. For example, those of the ivy bee, *Colletes hederae* can contain 300 nests per m^2 (Bischoff et al 2005). Solitary bee ecology is highly diverse with species often having specific nesting and forage requirements. For example, in Germany 30% of bee species are classed as oligolectic (Westrich 1996), including *C. hederae,* whose foraging ecology is studied in Chapter Four.

Solitary bee species also often have limited flight distances which depend on their body size (Gathmann and Tscharntke 2002, Zurbuchen et al 2010a) meaning that their floral and nesting requirements need to be provided within a certain distance of their nest. For example, the large bodied *Hoplitis adunca*, which is similar in size to a worker honeybee, is capable of foraging at distances of around 1.4km. However, in one study over 50% of females did not travel further than 300m from the nest (Zurbuchen et al 2010a). This means habitat loss can be particularly detrimental to solitary bee populations. A direct example of how distance to forage can negatively affect a population is seen in Williams and Kremen (2007), with female *Osmia lignaria* producing enough offspring to guarantee a sustainable population when nests were surrounded by natural habitat with appropriate floral resources, whereas in sites where natural habitat was more distant from the nest (in conventional farmland) females were unable to produce enough offspring for population persistence. Zurbuchen et al (2010b) found that the proportion of brood cells provisioned per time decreased with increasing foraging distance for two studied solitary bee species.

In the UK, the average proportion of $1km^2$ grid cell occupancy for solitary bees has declined by an estimated 32% between 1980 and 2013, compared to an estimated increase of 38% for 21 species of eusocial bee (Powney et al 2019). There are currently 20 species of bee listed the UK Biodiversity Action Plan (UK BAP). Seven of these are bumblebee species, with the remaining 13 being non-eusocial solitary bees. Many of the solitary bee species on the list are restricted to only a few populations. For example, *Anthophora retusa* (Chapter Two) is only found in five sites all in the south of Britain. Others, such as *Eucera longicornis,* are slightly more common (Chapter Three). However, the range of this species is also now restricted to the south of Britain, although historic records state that it used to be located as far north as Yorkshire (Falk and Lewington 2015).

Although many solitary bee species are in decline, often resulting in small isolated populations, one recent colonist is increasing in range and abundance. *Colletes hederae,* the ivy bee, which arrived in southern Britain in Dorset in 2001, has increased its range with records of it now being located as far north as Carlise, c. 20km from the Scottish border (NBN Atlas). Although the exact reasons for its rapid range expansion are unknown, its reliance on a common plant species for forage, ivy, is thought to be a contributing factor (Chapter Four).

1.5 Knowledge gaps on solitary bee conservation

In the last 600 years 70 insect species have been declared globally extinct (Dunn 2005). However, due to missing research on certain insect groups this estimate is thought to be much lower than the actual value, with one estimate proposing 44,000 extinctions as a more realistic figure (Dunn 2005). In Europe, 2.4% of bee species are classed as critically endangered by the IUCN (Nieto et al 2014). However, again due to limited monitoring, this figure is an estimate, as for 57% of European species there is not enough data to evaluate their extinction risk (Nieto et al 2014).

A research bias towards certain taxa is a major issue for understanding bee population trends and the responses of different species to habitat and land use change (De Palma et al 2016). Some insect groups are heavily researched, with long term monitoring programmes and charities using citizen science to obtain large data sets on changing distribution and abundance. Examples include the Bumblebee Conservation Trust "Beewalks" scheme, and the Butterfly Monitoring Scheme. These programmes have been vital for obtaining data on population trends, along with increasing public knowledge on various pollinator species. In contrast, a disproportionately low emphasis on solitary bee research compared to eusocial bees can be shown with a simple Web of Science search. A search for bumblebees in the title or topic identifies 2,558 published journal articles between 2010 and 2021. The same search for solitary bees returns half this, with 1,288. This is of course a crude measurement, but it highlights a real discrepancy in research, especially when considering that over 90% of global bee species are classed as solitary. Similar results are seen for studies on honeybees. Wood et al (2020) conducted a similar search using Scopus, searching for papers on 75 genera of bees. They found 78.4% of the identified publications were studies on *Apis mellifera,* meaning that for the remaining taxa there was an average of just 1.3 publications per species (Wood et al 2020). This gap in research makes it difficult to both identify population trends and to

understand why certain species may be declining. Take the rare solitary bee *A. retusa*. To help conserve the populations in the small number of areas where it is found requires knowledge of factors such as its foraging and nesting requirements. However, this information is currently scarce (Chapter Two).

Even the more heavily studied insect groups, such as butterflies, have examples of where a lack of knowledge on a species ecology has almost resulted in a species extinction, for example, the large blue butterfly (*Maculinea arion*). For most of its documented history in the UK, *M. arion* populations were in decline (Thomas 1995), with the reasons not entirely understood. In 1972, when numbers were critically low, an intensive study on the species ecology was conducted to identify the key information needed for its conservation. Although the study was too late for the UK population, which became extinct in 1979, the information on its ecology was eventually used to help manage reintroduced populations. By implementing conservation and management strategies using the ecological information gained from the previous research, the UK population has since recovered with the species now located in 33 sites in the South of England (CEH 2008). This is a key example highlighting how important studies examining the ecological requirements of species are in ensuring effective conservation.

Not only is knowing the ecological requirements of species needed for conservation, but also educating the public on the importance of pollinators. This is key to gaining support for conservation measures, for example, purchasing bee friendly flowers (Wignall et al 2019), or not calling exterminators to remove harmless nesting bees (Appendix A). However, there is still little knowledge and research on the 250 solitary bee species in the UK. This lack of public knowledge on British bee diversity was highlighted in a survey by Royal Mail, with more than half (53%) of participants unable to name a single species of bee (The Guardian 2015).

1.6 Impacts of weather on foraging

Understanding species specific requirements, such as foraging ecology, is an essential part of conservation. However, understanding foraging behaviour more generally is also of importance both as an essential part of the biology of bees (and most animals) and also because of its relevance to conservation. Animals show many adaptations that enhance foraging effectiveness and efficiency (Stephens and Krebs 1986, Boyd et al 1997, De Knegt et al 2007), and when foraging face many challenges. These include the effects of abiotic

variation in their immediate environment (Porter and Tschinkel 1987, Soulsbury et al 2008), which can cause dramatic shifts in behaviour. For example, bald eagles do not forage in high winds and rain (Elliott et al 2006) and Atlantic salmon will switch from foraging during the day to the night in response to cold temperatures (Fraser et al 1995).

Changes in weather can influence the foraging ecology and behaviour of pollinator species in a variety of ways. For example, heavy rainfall will often cause insect foraging to stop altogether (Inoue et al 1985, Joshi and Joshi 2010, Lawson and Rands 2019). However, some bee species are known to continue foraging in light rainfall, although this may compromise their foraging efficiency: *Bombus terrestris* workers collect less pollen on wet days (Peat and Goulson 2005). Honeybees do not forage in temperatures below approximately 10˚C (Heinrich 1996) and changes in ambient temperatures can influence floral choice (Norgate et al 2010). These studies all highlight how influential changes in environmental conditions are for foraging bees and their behaviour, and how understanding these changes is becoming ever more pressing due to our changing climate. Studies assessing large scale changes to insect distribution and abundance in relation to climate change are becoming more common and are important. However, it is also important to understand how it influences behaviour on an individual level. For example, *B. terrestris* visitation rate decreases when foraging on *Sinapis arvensis* grown in drought conditions, due to decreased flower size and abundance (Kuppler et al 2021). Climate models predict higher global temperatures, droughts (IPCC) and increasing wind speeds (Zeng et al 2019). There are many studies examining how the effects of temperature and drought may influence bee species (Vogt 1986, Blazyte-Cereskiene et al 2010, Minckley et al 2013, Soroye et al 2020). However, studies on wind and its impacts on behaviour are limited.

1.7 The effects of wind on foraging bees

Wind is capable of both helping and hindering foraging animals. For example, the wandering albatross benefits from favourable tail and side winds whilst traversing the ocean (Weimerskirch et al 2000 & Weimerskirch et al 2002), whereas high winds cause leaf cutter ants to collect smaller sized pieces of vegetation when foraging, reducing the amount of food a colony can collect (Alma et al 2017). Wind poses significant challenges for many flying animals, especially insects, who regularly interact with the unsteady airflow and turbulence created by wind (Combes and Dudley 2009) and whose small body size, resulting in a larger

surface area to volume ratio, will make increasing wind speed a greater challenge than for birds, due to the increased effects of air resistance (Hunter 2007).

Studies examining the impacts of wind on foraging bees have often focussed on how it influences flight mechanics (Crall et at 2013, Ravi et al 2013, Chang et al 2016, Crall et al 2017) and how it decreases overall foraging activity (Pinzauti 1986, Vicens and Bosch 2000, Tuell and Isaacs 2010). For example, bumblebees are known to alter their body orientation in response to turbulence caused by wind (Ravi et al 2013) and honeybees will speed up when approaching moving obstacles when flying in wind (Burnett et al 2020). Depending on the flower species, honeybees can make an estimated 250 and 1446 flower visits per trip (Ribbands 1949, Goodwin et al 2011), many of these requiring a bee to fly between individual flowers or even plants. Even a small increase in the time taken to travel between and land on flowers caused by wind-induced delays would be multiplied hundreds or even one thousand times (Couvillon et al 2015) per hour of foraging. The results in Chapters Five, Six and Seven examine how increasing wind speed is capable of significantly reducing the foraging efficiency of bees foraging on both natural and artificial flowers, and how specific foraging behaviours are influenced.

1.8 The effects of wind on flowers

Not only does wind directly impact foraging insects, but it is also capable of indirectly influencing foraging behaviour through its impacts on flowers. Flowers will sway in the wind, with various adaptations and environmental factors influencing the extent of this motion (De langre 2008, Warren and James 2008). Flower and leaf movement in wind has been suggested as a possible mechanism to reduce damage from insect herbivory and pathogens (Yamakazi 2011, Warren 2015) highlighting the challenges flower motion may pose for foraging pollinators. However, moderate flower motion has also been found to increase pollinator visitation (Warren and James 2008).

Flowers also have morphological adaptations to make handling by bees easier when moving. For example, some plant species have flowers with conical shaped cells on their surface that assist flower handling through improved grip (Whitney et al 2009), which is potentially beneficial to pollinators in windy conditions when the flower itself is moving (Alcorn et al 2012). Flowers exhibit a wide range of morphologies which are expected to interact differently with wind and pollinators. For example, larger flowers may be easier to see and land on and flowers placed on long inflorescences are likely to sway more in windier

conditions (Warren and James 2008). Flower morphology is capable of influencing handling time in the absence of wind (Balfour et al 2013). However, no studies have examined differences in foraging efficiency on different plant species in the presence of wind. The effects of plant species and flower movement on the foraging behaviour of bees is covered in chapters Six and Eight.

1.9 Aims and objectives

Pollinator species are currently experiencing multiple challenges and more work on species specific forage requirements, along with how the environment can alter foraging behaviour, are needed. The aims of this thesis are 1) to increase knowledge on the forage requirements and behaviour of three solitary bees to aid in their conservation and 2) to understand how wind can influence the foraging behaviour of honeybees and bumblebees.

Chapter Two: Population assessment and foraging ecology of the rare solitary bee *Anthophora retusa* at Seaford Head Nature reserve.

2.1 Abstract

Anthophora retusa is a rare solitary bee which has declined throughout Britain and other European countries since the 1990s. It is thought to be restricted to five sites in Britain. However, information on these remaining populations is limited. Knowledge on population size, habitat and forage requirements and foraging distance, are important for successful conservation of the species. The population of *A. retusa* at the Seaford Head Nature reserve in East Sussex was surveyed. Transects within the reserve were conducted and population estimates using mark recapture were made for 2018 and 2019. Pollen from foraging females was analysed alongside visual sightings to determine forage requirements. The total population was estimated to be 91 in 2018 (males and females) with an estimated male population of 167 in 2019. The most visited flower species by females was *Glechoma hederacea* (66% of visits) but flower preference changed throughout the flight season, shifting to Fabaceae species and *Iris foetidissima* with 16 plant groups identified in pollen samples. Bees were geographically restricted to a small area within the reserve (approximately 30ha). Although the exact location of nesting sites was not determined with certainty, it is thought nests are in the loess deposits at the top of the inaccessible sea cliff face. This project suggests the presence of appropriate nesting sites may be limiting *A. retusa* distribution as they appear to forage on common plant species. More research is needed on the exact nesting requirements of the species.

2.2 Introduction

Understanding the ecology of a rare species is vital in devising appropriate conservation strategies. Global examples of where this understanding has resulted in conservation success stories include the Asiatic lion in the Gir forest (Singh & Gibson 2011) and mountain gorillas in Bwindi national park (McNeilage et al 2006). Examples in the UK include the Large Blue butterfly (Thomas 1995) and the Eurasian bittern (Brown et al 2012).

In addition, information on population sizes and trends is required to determine if a species is rare, or if a population is small and at risk of extinction (Jackson 2019). Understanding these trends is becoming increasingly important in the current ecological climate with declines reported in many species globally (Thomas et al 2004; Beebee & Griffiths 2005; Goulson et al 2008; Winfree et al 2009; Potts et al 2010). Species extinctions begin with the loss of local populations. Smaller, isolated populations are at increased extinction risk (Purvis et al 2000), especially in species with intermediate mobility (Thomas 2000 & Traill et al 2007) or that have specific habitat or resource requirements (Thomas 1995).

In the last 600 years 70 insect species have been declared extinct globally (Dunn 2005). However, this number is thought to be much less than what is expected due to lack of research on certain insect groups, with one proposed estimate of 44,000 species extinctions (Dunn 2005). Globally, pollinator species are in decline (Biesmeijer et al 2006 & Potts et al 2010). In Europe, 2.4% of bee species are classed as critically endangered by the IUCN (Nieto et al 2014). However, again due to limited monitoring, this figure is an estimate, as for 57% of European species there is not enough data to evaluate their extinction risk (Nieto et al 2014). An example of a bee species thought to have recently gone globally extinct is *Bombus franklini.* It had a narrow distribution, only ever recorded in an area approximately 240 by 110km in southern Oregon and northern California in the United States (National Research Council 2007). For bees, the threat of extinction is not just limited to *B. franklini*, with four other *Bombus* species in Northern United States being placed on the IUCN at risk list for pollinators (National Research Council 2007).

Globally there are an estimated 20,000 species of bee. Of these, 250 are bumblebees (Goulson 2008), approximately 300 are stingless bees (Francisco & Arias 2010) and nine are honeybees (Koeniger and Koeniger 2000). Most of the remaining c. 19,500 are not eusocial and are often called solitary bees. There are many studies examining bumblebee species declines (Goulson

et al 2008, Cameron et al 2011, Meeus et al 2011) in the UK and elsewhere. However, studies on solitary bee species are minimal.

In Britain 12 species of bee have gone extinct since 1851, of which 2 were bumblebees, although no extinctions have been reported since 1990 (Ollerton et al 2014). However, from 1980 to 2013 British solitary bees have suffered a 32% decline in average occupancy (Powney et al 2019). Declines of both bumblebees and solitary bees in the UK are thought to be due to a variety of factors including habitat destruction, intensification of agriculture (Buchmann & Ahrne 2005), reduced growing of clover due to the invention and use of nitrogenous fertilizers (Ollerton et al 2014) and pesticides (Goulson et al 2015 & Woodcock et al 2017).

Solitary bees are not only an important component of biodiversity, but also contribute to pollination of both crops (Klein et al 2003, Ricketts et al 2004, Greenleaf and Kremen 2006, Holzschuh et al 2012) and wild plant species (Ollerton et al 2011, Rollin et al 2013). Solitary bees are at risk of local extinctions due to their often-limited foraging distances (Gathmann & Tscharntke 2002, Greenleaf et al 2007), specialised nesting (Westrich 1996; Wcislo & Cane 1996; Zurbuchen et al 2010) and forage requirements (Westrich 1996, Wood et al 2017). Determining these requirements, such as plant species on which they forage and where they nest, are likely to be important in making habitat management effective.

Anthophora retusa, the flower potter bee, is a spring- flying (active from April to late June), large (forewing length 8.5-10mm, Falk & Lewington 2015) solitary bee. It used to be widespread in southern England but since the 1990s it has greatly declined and is now restricted to a few sites (Table 1). It is thought that the species has become regionally extinct in eastern England as the last records from previously reported sites in Norfolk and Essex were in the early 1970s (Jackson 2019). The sites where it is still found are geographically small (Table 1). For example, in Dorset *A. retusa* are found nesting and foraging in a quarry site of approximately 30ha.

This decline of *A. retusa* is thought to be due to the intensification of landscape for farming and loss of nesting habitat as well as threats from flooding and cliff erosion (Evans and Potts 2004). It is a UK BAP priority species and is listed as Endangered (Falk 1991). Although assigned as Least Concern at the European level by Nieto et al. in 2014, due to it being widespread and common in the south of its range, the species has also been red listed in eight out of the 20 countries where it is found. Within these eight it is regarded as critically endangered in Estonia and endangered in the Czech Republic and the Netherlands, but there is

very little information on individual populations. The declines mirror that of some bumblebee species (Edwards and Jenner 2008).

A geographically restricted population of *A. retusa* was identified at the Seaford Head local nature reserve in East Sussex in the 1990s by Edwards and Jenner (2008). The aims of this current study were to determine several important ecological parameters for this population. First, to determine the population size using mark recapture. Second, to determine their main sources of forage using both pollen analysis and observations of individual foragers. And third, to identify its foraging range within the study area at Seaford Head using transect surveying of bees on flowers. In addition, this information was used to estimate foraging locations and distances travelled when foraging.

Table 1: Both historical and current locations of where *A. retusa* has been identified and the date of last recording. Where known, the habitat type and more detailed information on the population is described.

County and Site	Last recorded	Status	Habitat type	Population description	Reference
East Sussex Seaford Head	2020	Confirmed Present	Coastal grassland, shrubs and chalk cliff	Thought to be nesting in the sandy loess deposits on the cliff face above chalk. Total area where they are observed foraging 30ha.	This study
Dorset Hanson Quarry	2019	Confirmed Present	Heathland	Nesting aggregation in wind-blown sand area on the edge of a quarry pit site. Total area c.30ha with individuals seen foraging at multiple locations surrounding the pit.	Pers. Obvs GH
Dorset Lulworth ranges	2005	Thought to be present	Coastal	As the land is occupied by the Ministry of Defence surveying is difficult. However, bees have been found throughout the ranges	Bug life report Pers. Comms Mike Edwards
Isle of Wight Culver Down	2002	Thought to be present	Coastal	Restricted to a small area of coastline, less than 1km radius	Pers comms. Adam Wright
Bristol	1920	Considered lost	-	-	Horsley et al 2013
Somerset Milbourne Port	1839	Considered lost	-	-	Horsley et al 2013
Norfolk	1970	Considered lost	-	-	Jackson 2019
Bedfordshire	1946	Considered lost	-	-	Jackson 2019
Suffolk	1899	Considered lost	-	-	Jackson 2019
Essex	1970	Considered lost	Coastal, low grassland	-	Jackson 2019
Hampshire	2000	Status unknown	-	-	Hampshire biodiversity action plan 2000

2.3 Methods

2.3.1 Study site

Seaford Head Local Nature Reserve is located east of Seaford town and is bordered to the south by chalk sea cliffs and to the east by the Cuckmere River estuary. It is within the Sussex Downs Area of Outstanding National Beauty and is jointly owned by Seaford Town Council, the National Trust and the East Sussex County Council. The landscape consists of

coastal scrub, pasture grazed by sheep and cattle and both grazed and ungrazed grassland rich in wildflowers. It is classed as a nature reserve and SSSI (Site of Special Scientific Interest. Natural England 1999) due to the presence of nationally rare species, including *Anthophora retusa*, *Adscita statices* (forester moth) and *Seseli libanotis* (moon carrot) (Sussex Wildlife Trust). There is a diversity of habitat types throughout the reserve. The section of the reserve where the survey was conducted consists of a mixture of scrubland and occasionally grazed grassland bordered by permanent pasture (Fig 1a). It is the only part of the reserve where the cliffs have exposed loess deposits, highly porous silt sediment formed by the accumulation of windblown dust (Frechen 2011). The western section is predominately scrubland bordered by the Seaford golf course (Fig 1b). The eastern side is low-lying meadow with the Cuckmere river flowing through the centre (Fig 1c).

The total survey area (Fig1a) was 25ha, approximately 30% of the 83ha reserve. Within this, land was classified into three habitat types (scrubland, cliff edge and grassland) and 11 fixed survey-transect routes were established. The survey area was chosen as a previous study by Edwards and Jenner (2008) and surveys conducted by Sussex Wildlife had identified the presence of *A. retusa* along the exposed cliff face and inland within the 25ha survey site. The site also ensured multiple habitat types could be included in the survey. Areas were classified as scrubland where species such as *Crataegus monogyna* (hawthorn), *Sambucus nigra* (elder) and *Rubus fruticosus* (bramble) were dominant and accounted for approximately 10ha with four transect walks (Fig 1a). Grassland was defined as areas with minimal or no tree cover, more than 100m from the cliff edge with wild flowers or low (less than 1.5m high e.g. Gorse) shrubs as the predominant vegetation. This accounted for approximately 10ha and contained five transects. The remaining 5ha was classified as cliff edge with wild flowers and grass the predominant vegetation and was any area than fell within 100m of the cliff edge and contained two transects (Fig 1a). Transects were made along pre-existing paths with adjacent flower patches being surveyed.

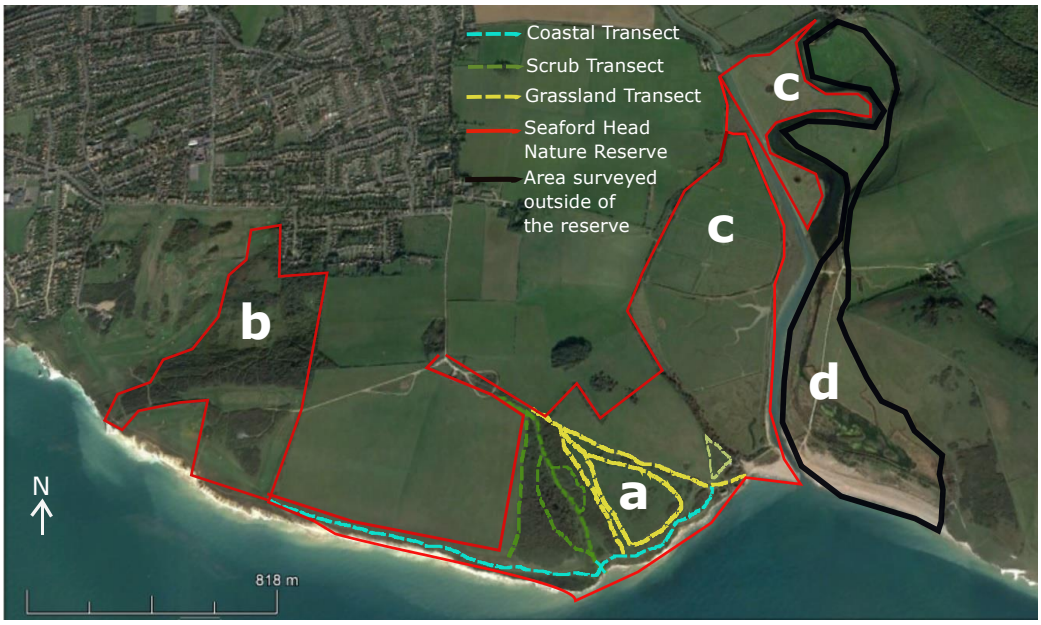


Fig 1: Survey area within the Seaford head nature reserve and all transects walked grouped by habitat type. A; the 25ha survey area, B; the western side of the reserve, C; the eastern side, D; area outside of the reserve where additional surveys occurred in 2020.

2.3.2 Mark-recapture and transect-survey walks

Mark -recapture data were collected 4 May to 18 June 2018 and 15 April to 22 June 2019 as these dates covered the period of peak activity for *A. retusa* (Falk and Lewington 2015). Surveys were adjusted to start earlier in 2019 to increase the survey time when males were active. Surveys were ideally made every two or three days but due to weather conditions not conductive to bee activity, sunny and 12°C or more, this was not always possible. The same transect routes were walked each survey day between 10:00 and 15:00 with the order randomised each day to minimise both time of day and weather effects on bee activity. Routes were walked once per survey day at a consistent speed with the same observer each day.

One challenge in studying *A. retusa* is that it cannot be differentiated on the wing from the very similar looking and abundant *A. plumipes* (Fig2a, 2b). Therefore, whenever either were seen they were caught with an insect net and identified. Females were identified by the colour of the hind tibial spur. In *A. retusa* this is red (Fig 2a) versus black in *A. plumipes.* Males of the two species could be identified because only *A. plumipes* has the obvious longer hairs on the mid tarsi (Fig 2c). Following species identification, *A. plumipes* were released. Any *A.*

retusa were released after being marked on the notum with individual colour-dot combinations using Revell© water based acrylic paints (Fig 2d, Zurbuchen et al 2010).

In addition, the flower species each bee was foraging on plus the GPS coordinates were recorded. If the bee was female, we attempted to collect a pollen sample. This was done by placing the female in a queen marking cage and using a fine toothpick to scrape grains from the scopa and the whole body into individual Eppendorf tubes which were frozen until analysis. Due to the rarity of the species, destructive sampling to ensure all pollen was collected from each female was not appropriate. Care was taken to remove all pollen from a female with the same technique of collection, as explained above, for each bee even if pollen did not appear to be present, to minimise the risk of only collecting larger or more visible grains. Occasionally pollen was not obtained from captured females either due to too few grains present or because the female was not collecting pollen. As a result, pollen samples were not collected on every survey day.

Using the GPS coordinates, the mean travel distances between the initial capture location and recapture points were calculated (Peakall & Schiestl 2004). If multiple recaptures of the same individual occurred (5 individuals in 2019 and 3 in 2018) then the mean distance travelled was calculated using the distances from the first capture point to all subsequent capture locations. In 2019 a large area of kidney vetch, *Anthyllis vulneraria,* was located along the cliff face. On 31 June, individuals foraging on this patch were caught using a net with a telescopic handle, marked and pollen samples taken from females. This was the only day where the patch was surveyed as it had not been previously identified and, therefore, to maintain a constant survey effort was not added to the survey route. To determine if female *A. retusa* had habitat preferences the average number of captures between the different habitat areas and transects were compared.

.

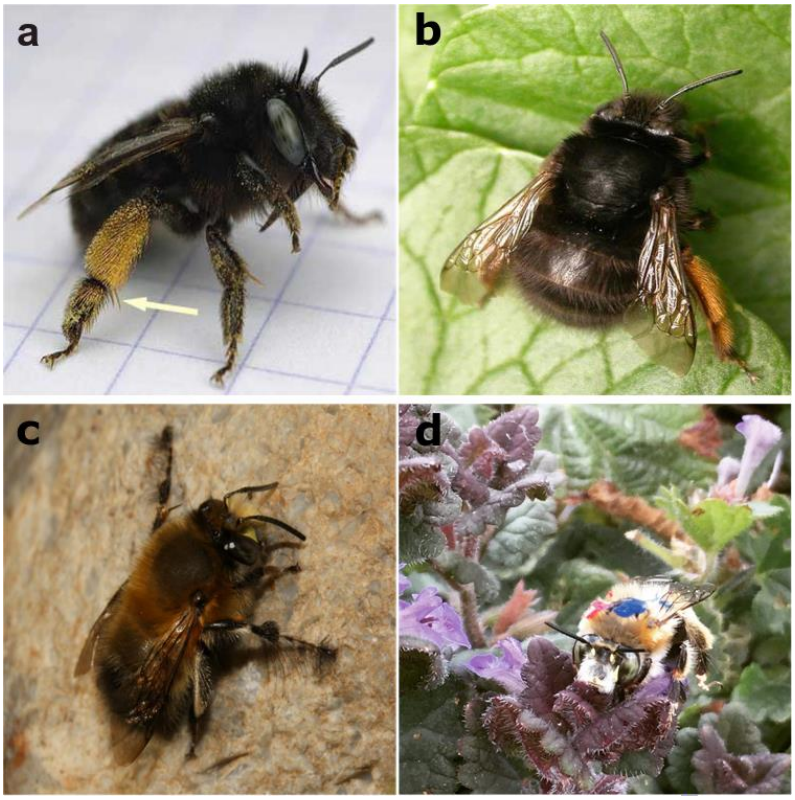


Fig 2: A; Female *A. retusa* with arrow indicating the red hind tibial spur (Aubert 2013) B; Female *A. plumipes* resting on a leaf (Jones 2013) C; Male *A. plumipes* with the distinctive hairy feet (Owens 2011) which does not occur in in *A. retusa* D; Paint-dot-marked male *A. retusa* foraging on ground ivy, *Glechoma hederacea*

Additional surveys were conducted outside of the core 25ha study area to determine if *A. retusa* was present in a nearby location where it has previously been found (Falk pers. Comms) and areas adjacent to the study site. On 28 May 2018, a survey was conducted inland from Seaford Head through the Cuckmere valley for approximately 4.5km, ending at coordinates 50.791003, 0.156665 (See Appendix B1). In 2020, additional surveys within Seaford head reserve were completed. These occurred in the western side which contains Seaford golf course (Fig 1b) and the surrounding wetlands to the east on both sides of the river (Fig 1c) along with the original 25ha survey area (Fig 1a). Outside of the reserve the wetlands on the east side of the river (Fig 1d) ending at coordinates 50.774232, 0.152147 and an area where *A. retusa* were once recorded, High and Over (coordinates 50.789516, 0.140185, Edwards per comms.) were also surveyed.

Each area was visited a total of three times, on days of good weather (above 12°C and dry) between May 27 and June 8 2020 during the female foraging period. Mark recapture and pollen collection were not conducted during these additional surveys.

Surveys additional to our study were made by Graeme Lyons of the Sussex Wildlife Trust in the western side of the nature reserve in 2016 and 2018 (Fig1b) and inland along the Cuckmere river for approximately 2km (2018, Fig1c).

2.3.3 Population size models and estimates

To estimate population sizes for males and females in both 2018 and 2019 the POPAN model of the Jolly-Seber method for open populations (Schwarz and Arnason 1996) was used in the programme "Mark" (version 6.2). Separate models were made for males and females for both years as previous work suggests separate models for sexes gives more accurate predictions (McKnight & Ligon 2017). Multiple models with different parameter criteria were created. The parameter index matrices (PIMs) used in the POPAN method include; Φ (the apparent survival rate), p (capture probability), pent (probability of entry into the population) and N (super-population size). Each of these PIMs was tested as either time dependent or constant and the "Akaike information criterion" (AIC) was used to select the most suitable model. Goodness of fit testing was performed to ensure the data met the model assumptions.

2.3.4 Pollen identification

Pollen was mounted in glycerine jelly with basic fuchsin to stain individual grains (Wood et al 2018). Identification was under light microscope at 400x magnification. Due to the low number of pollen grains present in each sample it was possible to count the actual number of grains per plant species identified. Identification to species level was attempted. However, due to similarities among species within *Trifolium*, other Fabaceae and Apiaceae these were identified to either genus or family level. Samples were grouped by date collected. The percentage of pollen grains from each plant species was calculated from the total number of grains identified on each sample day. If multiple bees were sampled on a day, the data were pooled.

2.3.5 Flower-abundance transects

In 2018 the abundance of flower species on each transect was recorded using a modified DAFOR vegetation scale (Croxton et al 2005). For each survey day the species present on each transect and its abundance on a scale of 0 to five (0: absent; 1: rare; 2: occasional; 3:

frequent; 4: abundant and 5: dominant). The overall daily abundance for each species was calculated as the average of its abundance across all transects for that day. The average abundance during the flowering period was calculated only using dates where the species was seen to be in flower on at least one transect. These measures provide information regarding when certain species became abundant within the female flight period and which species were flowering consistently throughout. Average abundance during the survey period was calculated using the average daily abundance for all survey dates. The flower survey dates were 20 and 30 May and 5,11 and 18 June 2018 to overlap with female activity. To determine differences in plant abundances between habitats, the average abundance of the five most visited plant groups by *A. retusa* females were compared between habitat areas and transects. These groups either accounted for more than 20% of flower visits or were over 20% of the pollen grains identified.

2.3.6 Statistical analysis

To determine if there was a significant difference in when peak activity occurred between males and females Mann-Whitney U tests were completed for both years separately due the difference in survey start date between years. To test for differences in plant abundance and the number of females captures between habitat types and transects Kruskall Wallis tests were run. To test for differences between survey years and sexes in distances between capture points, t-tests were performed. All statistics were performed using R version 3.4.2.

2.4 Results

2.4.1 Population estimates

In 2018 50 males and 42 females were caught and marked on 13 survey days, from 4 May to 18 June (See Appendix B2) No males were caught after 6 June. The day with the most male captures was 5 May, with 10, and 13 June for females, with 12 captures.

In 2019 there were 19 survey days from 4 May to 13 June. A total of 160 males and 57 females were caught (See Appendix B3). As in 2018, the day with the highest number of male captures was earlier than for females (10 May vs. 6 June).

In both 2018 and 2019 males were on the wing significantly earlier than females (Mann Whitney test U, 2-tailed: 2018, $U = 1874.5$, $P < 0.0001$; 2019, $U = 6777$, $P < 0.0001$).

To obtain population estimates separate models were run for each year and sex. Using goodness of fit testing, models that best fitted the data were chosen. The models used are shown in Appendix B4. The estimated ratio of males to females in 2018 was 1.1:1.0. In 2019 the male population was estimated to be approximately 3.6 times greater than in 2018, increasing from 46.8 to 167.1 individuals (Table 2).

Table 2: Gross population estimates for each year and sex calculated using the models described in Appendix B4 The total number of individuals caught for each year and sex are shown. Due to zero recaptures of females in 2019 a population estimate could not be made. However, 57 females were caught that year.

Year	Sex	Population estimate	±SE	Total caught
2018	F	43.9	4.5	42
2018	M	46.8	4.2	50
2019	M	167.1	6.5	160

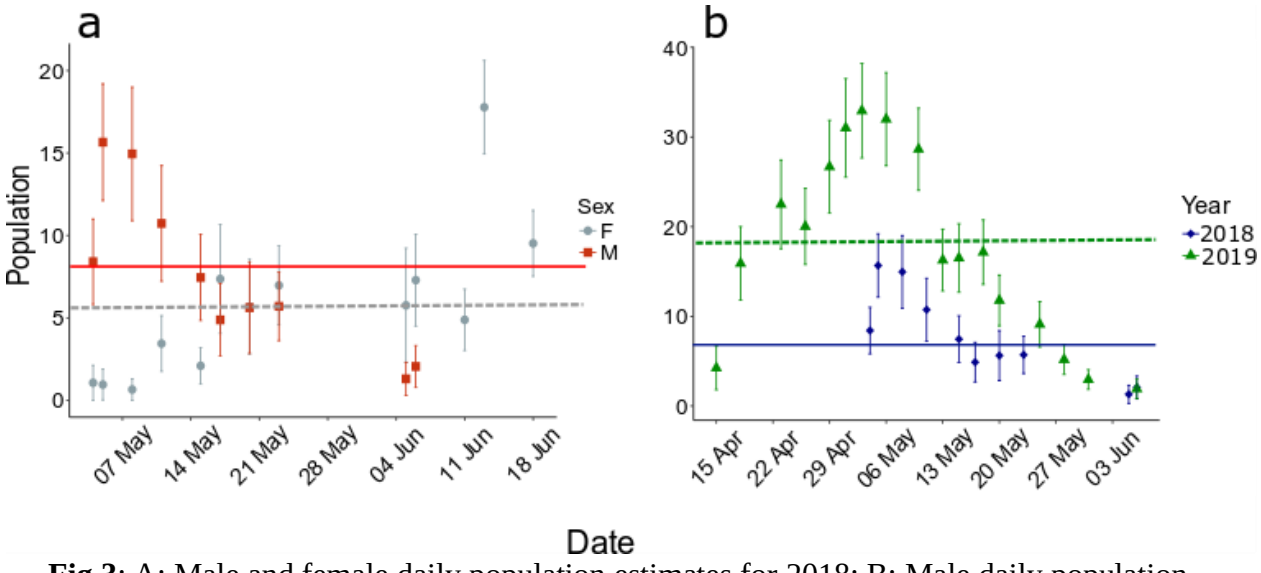


Fig 3: A; Male and female daily population estimates for 2018; B; Male daily population estimates for both 2018 and 2019. Standard error bars are shown. Horizontal lines indicate the overall mean of the daily population estimates. There were no daily population estimates for females in 2019 because zero recaptures occurred.

The daily population average for females in 2018 was 5.7 individuals and for males was 7.7 (Fig 3a).

In 2019 the male population daily maximum was 71% higher than 2018 and the daily minimum was 38% higher (fig 3b). The daily population average for males in 2019 was 17.3.

2.4.2 Pollen on females

In 2018 pollen was collected from 14 foraging females across the survey period. Pollen from 12 different plant species or families was identified. In 2019 pollen was collected from 12 foraging females with pollen from 14 different species or families identified. Pollen from ground ivy, *Glechoma hederacea* and Fabaceae species were the most common for both years. *G. hederacea* occurred in 64.3% (9/14) of samples and Fabaceae species in 50% (7/14) in 2018. In 2019 *G. hederacea* was present in 66.7% (8/14) and Fabaceae species in 75% of samples (9/12, Table 3). Nearly all females had pollen from at least two species or families (26/27) and pollen was often identified from plant species that *A. retusa* females were not seen foraging from, such as hawthorn *(Crataegus monogyna).* On average females were carrying pollen from 2.9 species.

Table 3: Plant species and families identified in pollen samples collected from foraging *Anthophora retusa* females in 2018 and 2019. All samples were collected from separate females. For plant species marked * females were not observed foraging on that species during the surveys. The mean number of pollen grains per sample was calculated only from samples where the species was present.

	2018			**2019**		
Plant Species	Pollen present on n/14 bees /(%)	Percentage of total grains collected from all females for year (%)	Mean number of grains per sample (±SE)	Pollen present on n/12 bees /(%)	Percentage of total grains collected from all females for year (%)	Mean number of grains per sample (±SE)
Glechoma hederacea	9/ (64.3)	32.1	7.0 ± 3.2	8/ (66.7)	34.1	54.6 ± 24.5
*Crataegus monogyna**	5/ (35.7)	13.3	5.2 ± 3.5	2/ (16.7)	0.5	3.0 ± 0.0
Symphytum officinale	2/ (14.3)	5.1	5.0 ± 4.0	1/ (8.3)	0.1	1.0
Fabaceae sp.	7/ (50.0)	13.3	3.0 ± 1.2	9/ (75.0)	22.4	31.9 ± 13.7
*Trifolium sp.**	4/ (28.6)	5.1	2.5 ± 1.2	5/ (41.7)	32.5	83.4 ± 80.9
Echium vulgare	1/ (7.1)	15.3	30.0	1/ (8.3)	1.6	21.0
Asteraceae sp.*	3/ (21.4)	3.0	2.0 ± 1.0	4/ (33.3)	5.3	17.0 ± 11.4
Atropa belladonna	2/ (14.3)	9.2	9.0 ± 7.0	1/ (8.3)	0.2	3.0
Iris foetidissima	2/ (14.3)	2.0	2.0 ± 0.0	1/ (8.3)	0.1	1.0
*Hippophae sp.**	1/ (7.1)	0.5	1.0	-	-	
Umbelliferae sp.*	1/ (7.1)	1.0	2.0	1/ (8.3)	0.1	1.0
*Acer pseudoplatanus**	-	-	-	3/ (25)	2.5	16.0 ± 15.0
*Filipendula ulmaria**	-	-	-	1/ (8.3)	0.2	3.0
*Rubus fruticosus**	-	-	-	1/ (8.3)	0.2	3.0
*Ranunculus sp.**	-	-	-	1/ (8.3)	0.2	2.0

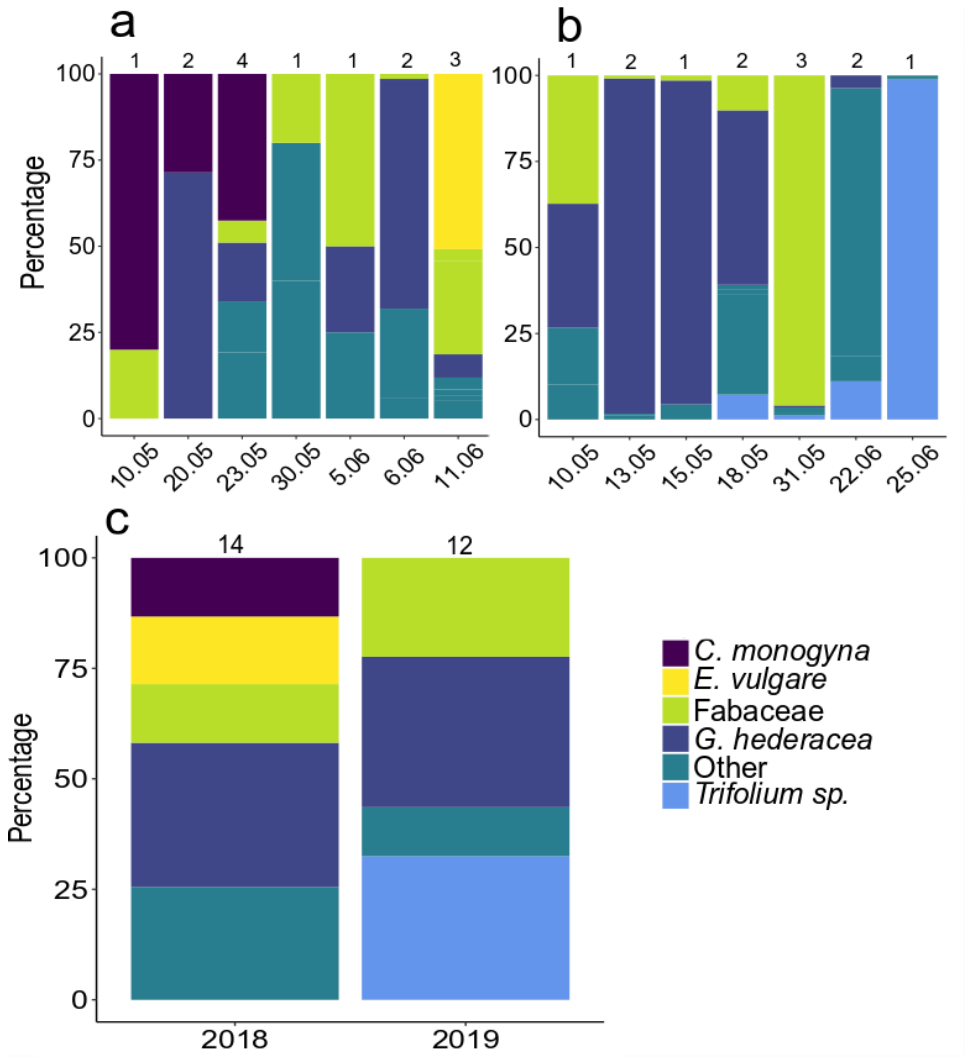


Fig 4: A; Proportions of pollen grains for all plant groups identified in samples in 2018 and B; 2019. C; All samples pooled for the year. Numbers above the bars indicate number of bees sampled.

In 2018 the four most prevalent plant species identified in pollen samples were *C. monogyna*, *G. hederacea*, *Echium vulgare* (viper's bugloss) and species of Fabaceae (excluding *Trifolium spp.*) (Fig 4a). *G. hederacea* was present in five out of the seven days sampled and species of Fabaceae were present in six. The date when plant species other than the top four most common were more prevalent was 30 May, with only 20% of pollen being from the Fabaceae and the remaining 80% from *Iris foetidissima* (stinking iris) and *Apiaceae* species (Fig 4a).

In 2019 the three most prevalent plant species identified in pollen samples were *G. hederacea* and species of Fabaceae and *Trifolium* (*T. pratense* and *T. repens*) (Fig 4b) *G. hederacea* pollen was the most common being present in samples from six of the seven days. Other plant species were most common on 22 June with *E. vulgare* comprising 78% of the samples and *Ranunculus sp.* comprising 7% (Fig 4b).

When pooled over all sample days in 2018 *G. hederacea* pollen grains comprised most of the pollen (33%) followed by *E. vulgare* (15%), *C. monogyna.* (13%) and Fabaceae species (12%, Fig 4c). In 2019 *G. hederacea* pollen was the most common (34%) followed by *Trifolium* species (33%) and then Fabaceae species (22%).

2.4.3 Survey walks

On 2018 survey walks most females were seen foraging on *G. hederacea* (63%) followed by *I. foetidissima* (29%, Fig 5c). On seven of the nine survey days all females seen were foraging on *G. hederacea*. Females were only seen foraging on *I. foetidissima* from 6 June (Fig 5a). Data from 2019 females followed a similar pattern, with most females seen foraging on *G. hederacea* (69%), followed by *I. foetidissima* (20%). All visits to *A. vulneraria* were recorded on 31 May. This was due to a large abundance of *A. vulneraria* on the cliff face being accessed and 0 females seen elsewhere in the study area on this day. *A. vulneraria* was not found elsewhere in the survey area nor was it located in the 2018 surveys. Females were first seen visiting *I. foetidissima* on the same date as the previous year (6 June, Fig 5b).

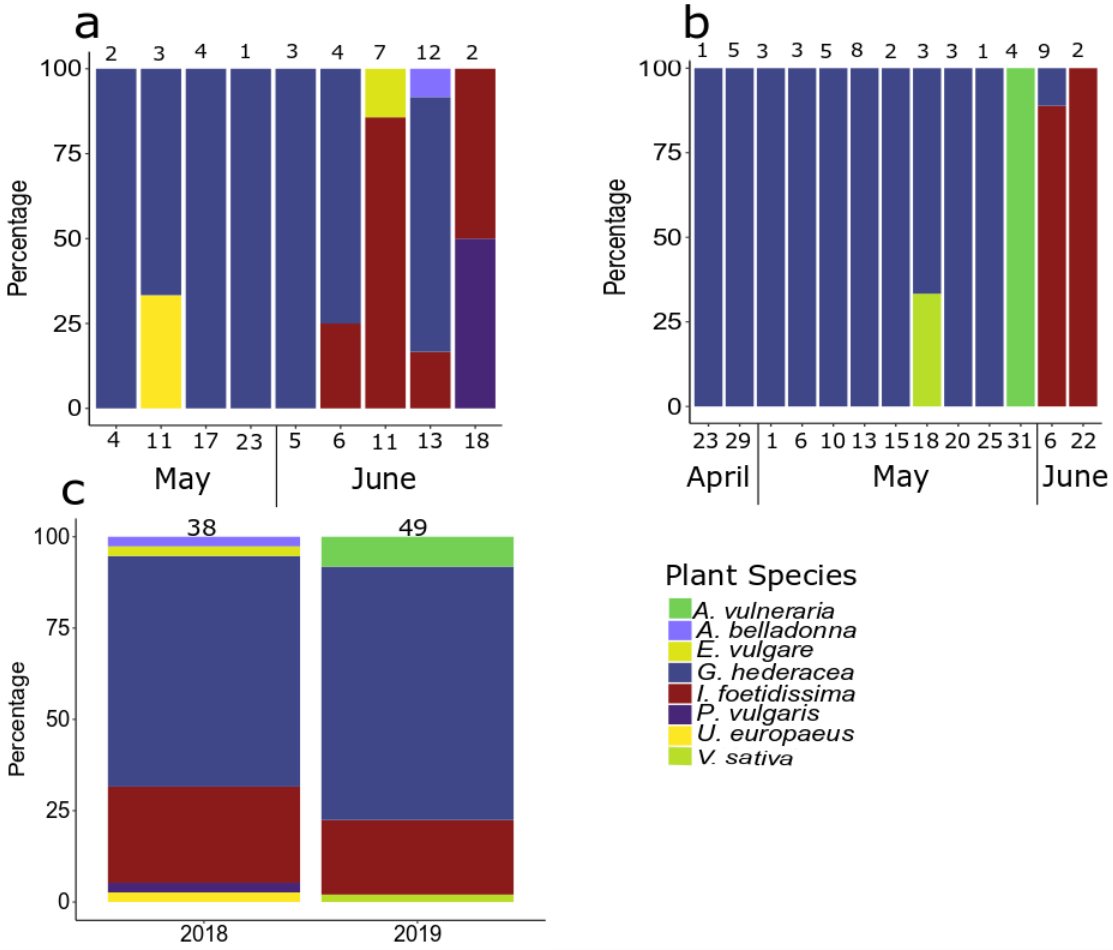


Fig 5: Percentage of females seen foraging on plant species during survey walks for each survey day in A; 2018 and B; 2019. May 28 2019 is not shown due to zero female *A. retusa* sightings. C; All sightings pooled for the year. The numbers above the bars indicate the number of bees.

In 2020 an additional 47.1km of survey walks were made outside the core study area. On the western side of the Seaford Head reserve (Fig 1c) 15.4km were walked in 4 hours with zero *A. retusa* seen. On the eastern side of the Cuckmere river, in the area of wetlands not within the Seaford Head reserve (Fig1d), 16.8km were walked in 4 hours also with zero *A. retusa* seen. In the wetlands located on the western side of the river within the reserve (Fig 1c) 7.4km were walked in 2 hours. A total of 24 female *A. retusa* were found within this area all foraging either on *G. hederaceae* (12) or *E. vulgare* (12). The furthest distance *A. retusa* was found outside of the initial study area was 250m. Beyond this zero *A. retusa* were located. Surveys within the core area in 2020 covered 6km in 4 hours with 26 female *A. retusa* identified. All were foraging on *I. foetidissma*. Surveys further inland at High and Over, a site

where *A. retusa* have historically been seen, covered 6.6km in 2 hours of surveying with no *A. retusa* identified.

2.4.4 Flower abundance

A total of 59 plant species were identified in bloom in the survey area (See Appendix B5). The average abundances and flowering periods for the five most visited plant species, either through observation or pollen samples, are in table 4. Of these *G. hederacea* was most abundant (mean abundance score 2.4). When including all flower species the most abundant on average across the survey period was *Ranunculus sp*. (Buttercups) (2.5). Although in flower throughout the entire survey period the average abundance of *G. hederecae* decreased each survey week, with its peak abundance score of 3.6 (on the first week of floral surveys, 20 May) declining to 1.4 on the last survey week (18 June,). *I. foetidissima,* the second most visited plant by female *A. retusa,* followed the opposite trend of not being in flower at the beginning of the survey period and reaching peak abundance on the only transect where it was found (score of 5) on 11 June.

Trifolium species (Kruskall- Wallis, χ^2=6.2, df=2, P=0.05) and *I. foetidissima* (Kruskall-Wallis, χ^2 =11.3, df=2, P=0.003) differed in abundance between habitat types. A post hoc Dunn's test showed that *Trifolium spp.* were significantly more abundant on the coastal transects compared to those in the scrub habitat (P=0.02) with no difference in abundance between grassland and coastal habitats (P=0.06). *I. foetidissima* was only identified along a single transect in the scrub habitat. When comparing species abundance between transects *I. foetidissima* and Fabaceae abundance differed significantly (*I. foetidissima;* Kruskall-Wallis, χ^2=41.7, df=10, P<0.001, Fabaceae;χ^2=29.1, df=10, P=0.001) but none of the other plant species tested did (See Appendix B6).

Table 4: A) The average abundance of the five most visited flower groups, those which were either more than 20% of pollen grains or accounted for more than 20% of flower visits by *A. retusa* B) the five most abundant plant species for all plants in the survey area and the dates where they were seen flowering. The average abundance was from all transects walked in the study area both for the dates when flowering and for the whole study period.

(A)			
Plant Species	Flowering dates	Average abundance throughout flowering period	Average abundance throughout study period
Glechoma hederacea	20.05.18-18.06.18	2.4	2.4
Trifolium sp. (2 species)	20.05.18-18.06.18	0.8	0.8
Echium vulgaris	11.06.18-18.06.18	1.0	0.4
Iris foetidissima	30.05.18-18.06.18	0.4	0.3
Fabacaea *spp.* (3 species)	20.05.18-11.06.18	0.9	0.9
(B)			
Ranunculus spp.	20.05.18-18.06.18	2.5	2.5
Glechoma hederacea	20.05.18-18.06.18	2.4	2.4
Ligustrum vulgare	20.05.18-05.06.18	2.8	1.7
Veronica chamaedrys	20.05.18-18.06.18	1.7	1.7
Lotus corniculatus	11.06.18-18.06.18	4.9	1.3

2.4.5 Spatial Distribution

Given the low numbers of recaptures in 2018, zero recapture of females in 2019 (See Appendix B2 and B3) and a non-significant difference in distances between years (t=-0.9, p=0.19) and sexes (t=-1.3, p=0.11), distance data were pooled for analysis. The average distance between the initial capture and subsequent recaptures was 122 ±21.2m (Fig 6) with most observations less than the mean (range 0-486m, mode 0-40m). When an individual had multiple recaptures, the mean distance travelled was calculated using the distances from the first capture point to all subsequent capture locations.

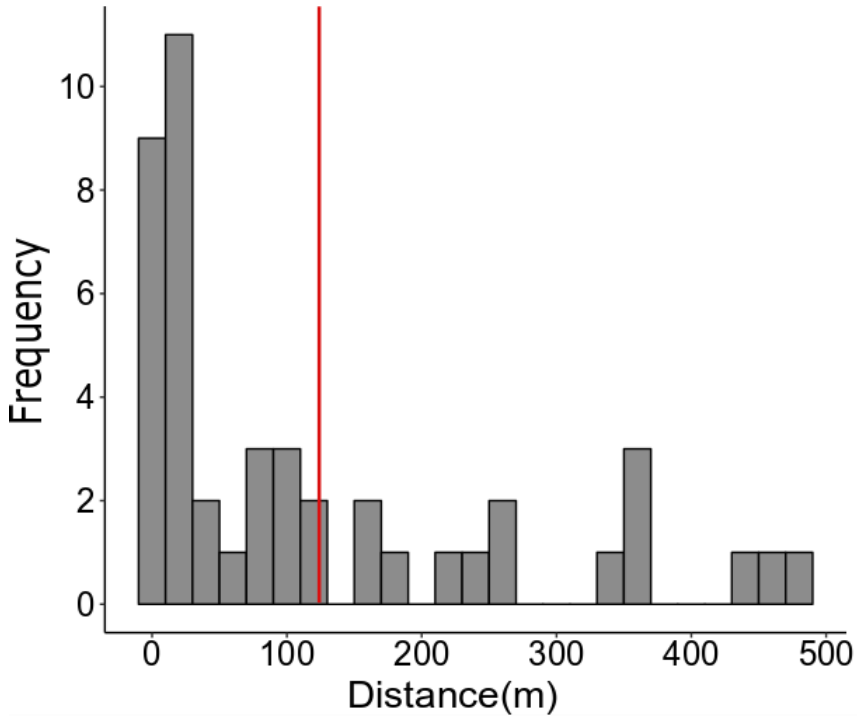


Fig 6: Frequency of distances between observations of individually marked bees (males and females) in both 2018 and 2019. Distances were calculated from the point of first capture to all subsequent captures. The red line indicates the average distance travelled, 122m. *N*=45

The GPS locations of caught individuals indicate foraging hotspots, especially for females. Clusters of females are in areas of either high *G. hederacea* density, or later in the season, *I. foetidissima*. In Appendix B8 the black rectangle indicates the transect (A1T3) where over the two years 43% of female captures occurred. This area accounts for only 5% of the whole study site with the average abundance of *G. hederacea* along the transect route being 3 (frequent) in 2018. It was the only transect where *I. foetidissima* was present, with an average abundance of 3.3 during its flowering period in 2018. There was a significant difference in the number of female captures between transects for both years (2018; Kruskall-Wallis, χ^2=36.3, df=10, P<0.001, 2019; Kruskall-Wallis, χ^2=27.5, df=10, P=0.002). A post hoc Dunn's test showed that transect A1T3 had significantly more female captures compared to all other transects for both years.

However, when examining capture rates between habitat types (scrub, grassland and coastal) there was no significant difference in 2018 (Kruskall- Wallis, χ^2=0.4, df=2, P=0.81) or 2019 (ANOVA, $F_{2,8}$=2.92, P=0.11).

A potential nesting area was identified in 2019 as males were seen patrolling along the cliff face and a mating pair was found a few meters from the edge. Due to the inaccessibility of the cliff face for safety reasons no individuals could be identified. However, females were seen flying up from the cliff face and small excavations, possibly nests, could be seen in the layers of loess deposits at the cliff edge.

2.5 Discussion

Our results indicate that the *A. retusa* population at Seaford Head is very small and geographically confined. Mark-recapture indicates that the male population in 2019 (the last year of population surveying) was less than 200 individuals, with bees not seen outside of the Seaford Head reserve, including in surveys conducted in 2020. The small size of the population is unlikely to be because *A. retusa* forages on rare plants or specialises on a narrow range of species, as is the case in some rare bees (Zayed & Packer 2007) and insect herbivores (Ellis et al 2019). The plant species that was predominantly used for foraging was ground ivy, *G. hederacea*, which is extremely abundant in the local area and nationally. One important missing piece of information in our study is that we were unable to locate and study nest sites. However, it is likely that nesting was in windblown, loess deposits of soil and sand on top of the chalk, which is vertically exposed at the cliff face (Edwards and Jenner 2008, Horsley et al 2013). This deposit is an unusual feature of the cliff at Seaford Head and is not found, for example, on top of the chalk cliffs of the Seven Sisters and Beachy head, which run to the east of Seaford Head (Sussex Wildlife Trust).

Small isolated populations have a high risk of local extinction due to a wide range of factors including habitat loss, weather, demographic stochasticity and genetic influences (Shaffer 1981, Lande 1993). Coastal erosion is an accelerating process at Seaford Head, with an increase from 2-6cm/y^{-1} from the Holocene to 22-32cm/y^{-1} in the last 150 years (Hurst et al 2016). Frequent cliff falls may threaten nesting sites if these occur on the cliff face, for example in 2017 50,000 tonnes of cliff fell in one location along the edge (BBC 2017). A fall that large could result in a big proportion of nests being destroyed, particularly if they are aggregated. Although major cliff falls are a threat to nesting aggregations, erosion is a natural and important process for cliff nesting species (Evans & Potts 2004).

Male population within our survey period reached its peak approximately a month earlier than the female peak, indicating that *A. retusa* emergence is protandrous, a common

characteristic of solitary bee species and many other insects (Eickwort & Ginsberg 1980). Mark-recapture showed a 255% increase in the estimated gross male population from 2018 to 2019. This may have been in part due to a longer survey period in 2019, which began earlier and therefore encompassed more of the male's flight period. However, the daily population estimates also increased, more than doubling, from an average of 7.7 in 2018 to 17.3 in 2019. The change in female population between the two years is unknown because zero females were recaptured in 2019. The reason for this is not known. It might be due to the paint marker used rubbing off the females as, unlike males, they are involved in nest excavation, increasing this chance of removal. However, the same paint brand was used for both years and is used in other mark recapture studies (Zurbuchen et al 2010). Another explanation could be that, later in the flight season, females were restricting their foraging activity to a large patch of *A. vulneraria* located along the cliff face. Multiple females were seen foraging here and were inaccessible for safety reasons. Therefore, if marked it was not possible to recapture them. These flowers were not discovered in 2018, therefore it isn't known if females were foraging on them to the same extent. A final explanation could be the population is much larger than expected. However, the sex ratio in 2018 was approximately even, 1.1:1.0F, which is expected on evolutionary grounds (Fisher 1930). Therefore, a much larger female population compared to the male population in 2019 is unlikely.

Sufficient and appropriate forage is vital for population survival and persistence (Schultz & Dlugosch 1999). *G. hederacea* was the major floral resource for female *A. retusa*, especially at the beginning of their flight season. Over both years a total of 16 different plant species and families were identified in pollen samples, with *G. hederacea*, *E. vulgare* and Fabaceae species being the most common, indicating that *A. retusa* is polylectic (Müller & Kuhlmann 2008). In 2018 *G. hederacea* was the most prevalent pollen (was 34% of all pollen grains identified) and in both years was the most popular, representing 73% of all flower visits recorded during transects (Fig 5). The second most visited plant species was stinking iris, *I. foetidissima* (23% of flower visits). However, pollen from *I. foetidissima* was only found in 12% of samples (3/26 bees) and comprised less than 1% of the total grains identified across both years. This perhaps suggests that *I. foetidissima* is more important as a source of nectar than pollen. No males were seen foraging on *I. foetidissima*. However, there were very few males still foraging during the flowering period of this plant species. Female foraging preference was flexible, following changes in flower abundance, primarily the reduction of *G. hederacea* and bloom of *I. foetidissima*. The lack of visual sightings on some of the plant

species identified in pollen samples may be due to inaccessibility of certain parts of the survey site, with *A. retusa* foraging on these species in places where surveying was not possible (such as through dense scrub or on the very edge of the cliff face). These results indicate that the presence of particular flower species may not be as important as forage availability from a succession of species across the flight season.

The location of forage may also be important. Although the number of female captures did not differ between habitat types, one transect, in the scrub habitat, did have significantly more female captures than all others (see Appendix B8). Surrounding vegetation sheltered this transect from the wind. In addition, it contained abundant *G. hederacea* and later in the season was the only transect where *I. foetidissima* was found. Flower abundance has previously been found to influence bee foraging patterns (Goulson 1999, Hegland and Boeke 2006). Only *I. foetidissima* and Fabaceae species abundance differed between transects, with *I. foetidissima* abundance also differing on the larger scale of habitat type, along with *Trifolium* species. This lack of a larger scale differences in flower abundance of the most visited plant species by *A. retusa* may explain why the number of female captures did not differ between habitats, as the floral composition was relatively similar throughout the survey site. The higher number of captures on the single transect with the presence of *I. foetidissima* may indicate its importance as a floral resource.

These hotspots of *A. retusa* activity may also be due to individuals travelling only short distances to forage and seek mates. Most individuals (male and female) were caught less than 30m away from their initial capture. Being a large bee species (forewing length 9-10mm for females and 8.5-9mm for males, Falk & Lewington 2016) *A. retusa* should have the ability of foraging at moderate to large maximum distances (c. 700m or more), given that foraging distance correlates with body size (Gathmann & Tscharntke 2002, Greenleaf et al 2007).

Hofmann et al 2020 found significantly larger maximum flight distances compared to observed average flight distances in six solitary bee species, with maximum estimates up to 7 times larger. This is thought to be due to average flight distance being context-dependent, influenced by factors such as local resource availability (Zurbuchen et al 2010). If there are appropriate resources nearby to the nest, then females will reduce their energy expenditure and fitness costs by not travelling to flower patches further away (Zurbuchen et al 2010).

In this study *A. retusa* were found to have lower average flight distances than their estimated maximum, with an average distance of 122m. This agrees with the results found in Hofmann

et al 2020. For example, *Osmia cornuta,* a slightly smaller species than *A. retusa,* was found to have an average foraging distance of 107m with the maximum distance observed being 724m. The shorter flight distances seen by *A. retusa* may be due to the species being polylectic and that there was abundant forage found throughout the 30ha where they were observed in this study.

As the distances in this study were calculated between site of initial capture and subsequent recaptures, low distances may also be due to individuals showing site fidelity. Honey bees and bumblebee species are known to show site fidelity to a rewarding food source (Heinrich 1976, Comba 1999) where individuals will consistently travel to a patch of flowers even once they have finished flowering. *A. retusa* individuals were often caught either on the same patch of flowers or within a few meters as on previous survey days, perhaps indicating a similar behaviour.

Additional surveys outside of the core 25ha identified female *A. retusa* foraging on either large patches of *G. hedereceae* or *E. vulgare* within 250m of the original survey area. Beyond this point none were seen, perhaps indicating they are restricted to a specific area of the reserve (approximately 30ha). A mark recapture study on the rare solitary bee *Andrena hattorfiana* examined small location populations and the movement of individuals between them. Most populations contained fewer than 50 females, with the average distance travelled between plant patches often less than 50m (Franzén et al 2009), a similar finding to the distances seen by foraging *A. retusa*, with most individuals caught less than 30m from their original capture locations. On the western side of the reserve the habitat is predominantly scrub, with the Seaford Golf Course located throughout. The scrub is similar in floral composition to that found in the core study area, with patches of *G. hedereceae* present. However, the beginning of the scrub habitat where floral resources are most abundant is over 1km from the potential nesting site, with a large section of arable land between these points. In Franzén et al 2009 female *A. hattorfiana* rarely crossed areas where their pollen plant was not located, even if the distance was only 10m. The lack of captures of *A. retusa* in the areas surrounding the reserve indicate that perhaps the population at Seaford head is isolated and restricted due to females not wanting to cross patches of land with minimal or no forage available.

Since *A. retusa* forage on common wildflowers, why is the population at Seaford Head small, and why is this one of the few sites where it occurs in Britain? No definitive answer can be

given at this stage. Not only are floral resources essential for solitary bees but also appropriate nesting habitat (Franzén et al 2009). The population at Seaford head are thought to be nesting in loess, the sandy deposits exposed at the top of the cliff face (Edwards and Jenner 2008, Steven Falk 2019 pers comms.) with two mating pairs found along the cliff edge in 2019. There is little information on the historical populations of *A. retusa,* therefore understanding why they have been lost from other sites in Britain is not possible. The sites where they remain and where information is available appear to be mainly coastal and are either protected or managed by The National Trust or through nature reserve status (Culver Down &Seaford Head) or in an area with little human intervention that harms wildlife (Lulworth Ranges, MOD owned, Dorset Hanson quarry site). The coastal populations have access to chalk cliffs, like those at Seaford head, with the population at the Hanson quarry site having access to soft, sandy, disturbed banks. Other locations the species is thought to be currently found, and where it has been identified in the past, include heathland. Here the soil, although not formed from loess, shares similar characteristics such as being free-draining and sandy (Pywell et al 1994). More studies on these populations and locating the specific nesting aggregations is needed to help understand the nesting requirements and how this may be contributing to the species decline. Having preference for nesting sites is seen in other solitary bee species. Criteria include moist, compact soil with little or no vegetation (Wuellner 1999) or in other species soft soil on south facing slopes (Potts and Willmer 1997). These contrasting criteria in different species highlights the importance of understanding the species-specific nesting requirements.

Although there is still more information required on *A. retusa*, our data highlights the importance of conserving coastal habitats and the abundant wild flower species often found there. For *A. retusa* appropriate forage available in early spring, particularly. *G. hederacea*, and later in the species flight season the presence of *I. foetidissima* and Fabaceae appears to be important for the Seaford Head population.

Conservation success stories, such as for the Large Blue butterfly, with its successful reintroduction (Thomas 1995), give hope for rare species like *A. retusa* and emphasize the importance and value of studying local populations and the specific needs of different species. With it only found in a few, isolated populations, it is at great risk of extinction in Britain. This study has highlighted that more research is required on this species to fully understand the cause of its rapid decline and that the conservation of the sites where it is currently located is vital for the species survival in Britain.

Chapter Three: Population assessment and foraging ecology of nest aggregations of the rare solitary bee, *Eucera longicornis* at Gatwick Airport, and implications for their management.

3.1 Abstract

Eucera longicornis is a rare solitary bee that is in decline throughout its range in Britain and other European countries. Two nest aggregations that had been previously discovered in 2014 on land owned and managed by Gatwick Airport, Southern England, were studied in 2017, 2018 and 2019 to obtain information relevant to their conservation. Population estimates using mark recapture showed that the larger aggregation had a maximum population estimate of 659 individuals in 2018, with the smaller aggregation reaching a maximum population estimate of 221 in 2019. In terms of foraging requirements, microscopic analysis of pollen samples from females returning from their nests identified a total of 12 plant taxa across all years and both aggregations. Fabaceae pollen was present in 100% of samples and on average comprised 90% of each sample, indicating that the species is oligolectic. Both nest aggregations were on sloping banks of exposed soil arising from land management by the airport in 1999. Floral surveys of the landscape indicate that within 100m of both aggregations Fabaceae species are the most abundant due to the presence of a legume rich wildflower meadow alongside the adjacent river Mole, which was sown by Gatwick Airport. *Eucera longicornis* at Gatwick Airport is an example of how, if managed appropriately, industrial areas can provide valuable refuges for rare wildlife. Although no imminent risk of the populations going extinct, management and monitoring suggestions are provided to ensure these populations persist.

3.2 Introduction

Although the extent to which global insect declines are occurring is currently debated (Thomas et al 2019), there is no doubt that at many local scales some insect species are in decline (Didham et al 2020). More monitoring and research on population abundance is required to fully understand the state of the world's insects (Montgomery et al 2020). Examples of national declines include butterflies in the Netherlands, with 55% of common species suffering both declines in abundance and distribution between 1992 and 2007 (Van Dyck et al 2009). In the UK, 34/46 butterfly species declined in distribution area between 1970 and 1999 (Warren et al 2001).

Bumblebees, one of the best documented and most studied insect groups, have suffered ongoing losses in diversity in the UK (Goulson et al 2008) and Europe (Rasmont et al 2015). However, bumblebees are not the only groups of bees at risk. Between 1980 and 2013, solitary bees suffered an average 32% reduction in range (Powney et al 2019). In the UK, 23 bee and flower visiting wasp species have gone extinct since the late 1800s, with only two of these being bumblebee species (Ollerton et al 2014). For many solitary bee species, changes in landscape structure can be hugely detrimental as they often have specialised nesting, (Westrich 1996; Wcislo & Cane 1996; Zurbuchen et al 2010) and forage requirements (Westrich 1996, Wood et al 2017) alongside limited foraging ranges (Gathmann & Tscharntke 2002, Greenleaf et al 2007).

Some of the main drivers identified as contributing to insect declines are urbanisation and habitat loss due to agricultural intensification (Leather 2017). Despite covering a small area of the globe (~2%, Svirejeva-Hopkins et al 2003) urban landscapes have had detrimental effects on some insect species. For example, in a study examining bees and wasps in Belo Horizonte, Brazil, the abundance of stingless bees was negatively affected by the increase of buildings and loss of vegetation cover associated with urbanisation (Zanette et al 2005) and a study using allotments in the inner city of Stockholm found bumblebee diversity decreased with increasing urbanisation (Ahrne et al 2009).

However, in the Ahrne et al (2009) study they also identified that allotments with rich flower diversity can serve as important alternatives to natural habitats for some bumblebee species. This is seen in other studies, with some species readily utilising urban landscapes. Banaszak-Cibicka & Zmihorski 2012 found that some social bee species that emerged later in the year (June-July) were higher in abundance in the city centre compared to the surrounding

suburban area. In the UK, bee abundance did not to differ between urban, farmland and nature reserves, with bee species richness being higher in urban landscapes compared to farmland (Scriven et al 2013, Baldock et al 2015).

Some human-altered landscapes, such as brownfield sites, can become refuges for wildlife once abandoned. For example, in the UK, 194 invertebrate species of conservation importance have been recorded from brownfield sites. This includes 50% of rare solitary bees (Macadam & Bairner 2012).

Even in areas of semi-natural habitat where human disturbance is ongoing, management can be effective in maintaining, or even promoting, insect biodiversity. Areas under powerlines, for example, where regular clearing of forest must take place, were found to have a higher species richness and abundance of solitary bee species compared to areas of the same forest that have not been cleared (Steinert et al 2020). Sand quarries can also be important habitats for many invertebrate species (Buglife 2009) including some nationally rare solitary bee species (e.g. *Colletes cunicularis* Formstone & Howe 2013). Sites often blamed for environmental damage, such as airports, can be managed in a way to promote insect biodiversity (Kutschbach-Brohl et al 2010) and allow for the nesting of rare ground nesting birds (Kershner and Bollinger 1996). On semi-urban sites where rare species are located, it is important to conduct population monitoring and gain information on the specific ecological requirements of the species, to ensure the correct management scheme is conducted and effective (Kershner and Bollinger 1996).

Eucera longicornis, the long -horned bee, is a rare solitary bee that is a nationally declining species in the UK and is listed as a BAP priority species (JNCC 2007). It used to be widespread in England and Wales and was present in most of the counties south of Oxfordshire, with old records reporting individuals as far north as Yorkshire (Falk and Lewington 2017). It is now restricted to mainly southern sites, predominately coastal with a few inland populations as far north as Shropshire (Fig 1).

In 2014 *E. longicornis* was found to be nesting in a human-altered landscape on land owned and managed by Gatwick Airport by Rachel Bicker, the airports' biodiversity officer, in 2014 (Rachel Bicker, pers comms). There are two nest aggregations. One in a man -made soil heap approximately 200m from the runway, and the other roughly 1km north along the bank of the river Mole. Both are on land owned and managed by Gatwick Airport but outside the airport perimeter fence.

The Gatwick Airport *E. longicornis* are the subject of this investigation. The aims are to determine key information about the Gatwick population that could be used as a baseline for population monitoring and to provide information relevant to conservation. We determined the population size of each aggregation using mark recapture. We also determined the main forage sources through pollen analysis and surveyed floral resources in the surrounding landscape.

Fig 1: Current distribution of *E. longicornis* in the UK taken from the NBN atlas. The black star indicates Gatwick Airport, where this study takes place.

3.3 Methods

3.3.1 Study organism

E. longicornis is a medium sized solitary bee (fore wing length 9-10.5mm, Falk and Lewington 2015), the females slightly larger than the males. Males have the distinctly long antennae which give the species its name (Fig 2) and is unique in British bee species. *E. longicornis* is widespread throughout Eurasia, extending as far east as China (BWARS 2012). In the UK, adults emerge in May and are usually active until early July, sometimes extending into August. Females nest in the ground, normally in bare soil on south facing slopes in aggregations alongside other nesting females (Saunders 2014).

Fig 2: a: Female *E. longicornis* with paint mark foraging on *Lathyrus pratensis* at study site; b: Male *E. longicornis* foraging on *Symphytum,* showing the trademark long antennae.

3.3.2 Study Site

Two aggregations were identified in 2014 on land owned and managed by Gatwick Airport (Fig 3) in West Sussex, UK. In 1999, for flood management the nearby river Mole was diverted from the eastern side to the western side of a wood near the airport. Meanders were created to slow the flow and increase water retention within the floodplain. Raised areas were also created to divert water away from important infrastructure. Alongside the river wildflowers and legumes were seeded, resulting in a diverse and flower rich meadow. The work also resulted in a large amount of soil being taken from the river bed and piled into environmental bunds, which created slopes with scant vegetation cover. Natural erosion along the river bank also created areas of exposed soil.

3.3.3 Aggregation 1

The larger aggregation of *E. longicornis* is found along a slope about 200 meters north from the airport runway, latitude 51.150603 longitude -0.20474334 (Fig 3) and was first discovered in the spring of 2014. The aggregation is located on one of the steeply sloped mounds created in 1999 from soil excavated during the river Mole diversion. The mound is east facing, sparsely covered in short grass and bramble with patches of exposed soil (Fig 3). The aggregation extends over an area of approximately 140m^2. The landscape within the

surrounding 300m contains manmade structures, such as the runway, but also woodland and a wildflower meadow.

3.3.4 Aggregation 2

The other aggregation is located on the opposite side of the river, approximately 1km north east of aggregation 1 and backing onto Charlwood Park, grid reference latitude 51.159232 longitude -0.19627099 (Fig 3). It too is on a steeply banked slope, sparsely covered in short grass with patches of exposed soil due to natural erosion from walkers, approximately 10m from the river and facing south east (Fig 3). In 2018, the second year of data collection, nests were seen approximately 20m further along the bank to the west, in the direction of the airport. Due its proximity, it was considered to be part of aggregation 2 in both 2018 and 2019. In these years, the combined area containing nests was 120m^2, not including the gap between two nesting areas. The landscape within the surrounding 300m is similar to aggregation 1, with meadow wildflowers and dense woodland and manmade structures consisting of a road and carpark.

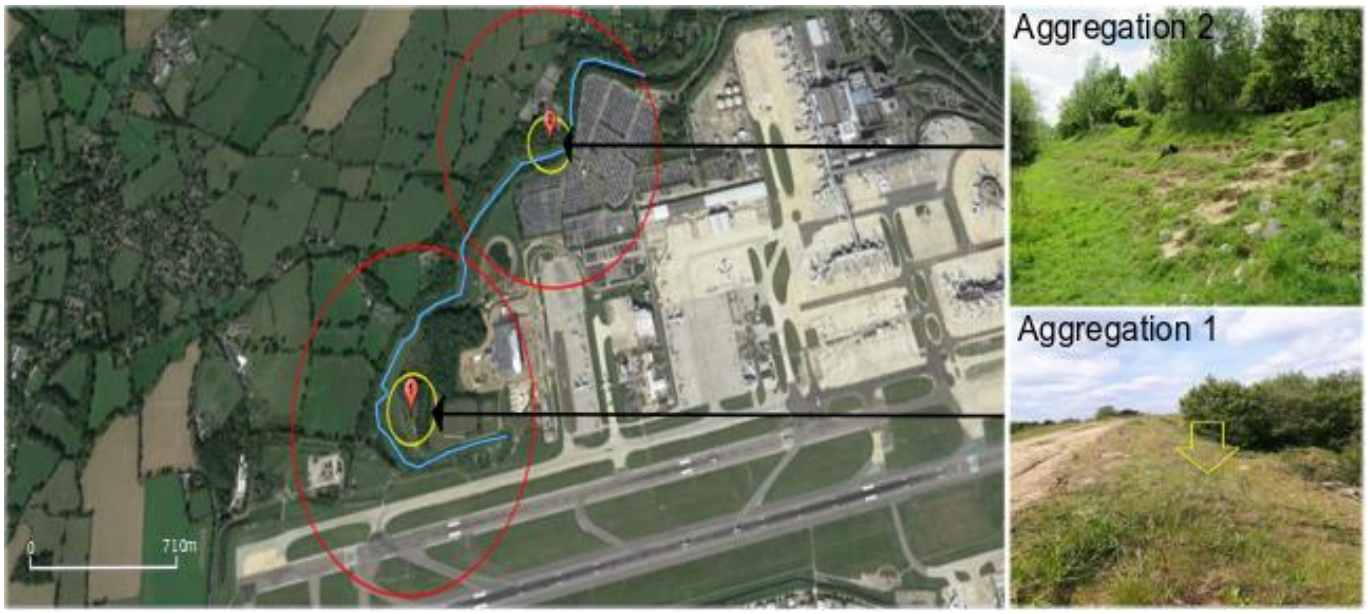


Fig 3: Location of the two Gatwick Airport *E. longicornis* aggregations studied. The yellow inner circle shows 100m from the centre of the aggregation, with the outer red circle showing 500m. The numbers indicate the aggregation. The blue line represents the river Mole. Photos on the right are of the banks where the nest aggregations are located (Rachel Bicker 2018)

3.3.5 Population estimates using mark recapture

Population estimates were made in 2017, 2018 and 2019 flight seasons using mark recapture. In 2017, data were collected in five study days per aggregation between 10 June and 1 July. In 2018 there were 8 study days, between 26 May and 11 July and in 2019 there were 10 study days between 21 May and 13 July. Field work took place only on days of good weather suitable for insect flight with temperatures from 17-30°C and sunny. Due to intermittent weather, field work could not always occur at consistent time intervals. Bees were captured at the aggregation using an insect net, starting between 10:00 and 11:00am and continued for four hours to cover the peak foraging period. An observer would walk through the entire aggregation and capture any returning or departing females or patrolling males seen. When caught the bee was transferred to a queen marking cage (Pixnor, China) and marked with an individual colour combination and pattern (Fig 2) using non- toxic acrylic paint (Revell, Germany). If the individual was a female carrying pollen, a small sample would be scraped from the scopa on each hind leg and placed in an Eppendorf tube for later analysis. Each sample was kept separate, and pollen was collected even if the female was a recapture. The bee would then be released.

3.3.6 Pollen analysis

From each sample 1mg±0.01mg of pollen was mounted in glycerine jelly with basic fuchsin to stain the individual grains. Identification used a compound light microscope at 200x magnification. The total number of pollen grains per plant species in the field of vision were recorded. Counts were taken from five random points on the microscope slide. The proportions of each species per sample were then calculated. Where possible, pollen was identified to species level using a reference collection from pollen collected from plant species at the study site, alongside a pollen identification book (Sawyer & Pickard 1981). However, due to similarities between some species this was not always possible and instead was identified to the lowest possible classification. For many Fabaceae this was to family level.

3.3.7 Floral surveys

In 2018, 4 floral surveys were conducted across the period of maximum female activity (on the 20 and 29 June, 4 and 10 July) to determine the availability of floral resources. The land within 500m of each aggregation was categorised into the following vegetation types: managed grassland (land actively managed by humans and the vegetation is predominately

grass); meadows (land minimally managed by humans, vegetation is predominantly tall grass with wild flowers); woodland (predominately trees); roadside verges (any land which falls directly beside a road). 500m was chosen as it falls near the maximum foraging range of female *E. longicornis* (Saunders 2014) and so is a relevant indication of potential forage.

Using a 50x50m grid and stratified random sampling, 100m transects in each vegetation type were made based on by the area of each land type (Table 1) and the number of replicate land type areas available (approximately 1 transect per 1.2ha). Due to access limitations, not all the surrounding vegetation could be sampled and, on some days, not all the 100m transects could be completed. Along each transect, the number of inflorescences per species were estimated. From this the average number of inflorescences per species per 100m was calculated both overall and per habitat type. If a foraging *E. longicornis* was seen during a transect it was caught, marked and the coordinates were recorded.

Within both 100 and 500m of each nest aggregation the percentage cover of each habitat type was calculated using Google Earth Pro. Here an additional landscape type, manmade, was included. This consisted of areas that were predominately concrete and had very little vegetation. These areas were not included in the floral surveys as they were inaccessible (e.g. the airport runway) but also would have been devoid of flowers.

Additional surveys searching for other aggregations in the area were conducted. These occurred along the banks of river Mole over 1km from aggregation 2 in both a north west and north easterly direction. Suitable sites (a slight slope covered in sparse vegetation) were examined for nest holes of appropriate size indicating nesting female *E. longicornis* or for returning females or patrolling males. Suitable banks nearby to aggregation 1 were also surveyed. These surveys occurred each year once at the beginning of the survey period to identify patrolling males and once again when females began to emerge. An additional survey also occurred in June 2020.

Table 1: Area of accessible land of each habitat type for each aggregation and the number of 100m transects per survey date

Aggregation	Vegetation Type	Area (ha)	Number of transects			
			20 June	29 June	04 July	10 July
1	Managed Land	6.10	5	5	5	5
1	Woodland	4.53	3	3	3	3
1	Meadow	5.92	7	7	4	5
2	Managed land	4.84	4	4	5	4
2	Woodland	4.61	4	3	4	4
2	Meadow	3.56	5	5	5	5
2	Roadside	1.12	1	1	1	1

3.3.8 Data analysis

Mark-recapture analyses for each aggregation and sex were calculated using the program MARK which uses maximum likelihood models to estimate population parameters (Cooch and White 2004). Because the data covered most of the flight season population closure was not a reasonable assumption as individual births and deaths were likely. Therefore, the open population POPAN parameterisation was used to estimate population parameters. POPAN calculates the apparent survival rate φ, the probability of capture P, and pent, the probability than an animal from the superpopulation enters the subpopulation (the individuals occurring in the study). Model assumptions were calculated using the programme RELEASE GOF (goodness of fit) within MARK. The most appropriate model was selected using Akaike Information Criterion corrected for small sample sizes (AICc, Burnham and Anderson 1998) testing whether time dependent (t) or constant (.) parameters were more appropriate for the population. To test if sex ratios were biased a binomial test was run on the number of males and females caught. To test if there was a difference in the proportions of species present in pollen samples a general linear model with a binomial and log link function was used. Statistical tests and graphs were created using R (R core team 2017).

3.4 Results

3.4.1 Population estimates

Over all three years a total of 432 females were caught at aggregation 1 and 236 at aggregation 2. In 2018 and 2019, 199 males were caught at aggregation 1 and 68 at

aggregation 2 (see Appendix C1 and C2). No males were caught in 2017 because the survey started after their flight period.

To obtain population estimates separate models were run for each year and sex. Using goodness of fit testing, models that best fitted the data were chosen. The models used are shown in Appendix C3. Aggregation 1 was estimated to have a larger population in all years and for both sexes (Table 2) and was highest in 2019 at 440 females and 92 males (Table 2). For aggregation 2, both female and male estimates were also highest in 2019 at 183 females and 55 males (Table 2).

For aggregation 1 female survival estimates (Phi) ranged between 0.91 and 0.98. For males, the highest survival estimate was in 2018, with 0.93 compared to an estimate of 0.36 in 2019 (See Appendix C4). For aggregation 2, female survival estimates ranged between 0.89 and 0.95. Again, for males the highest survival estimate was in 2018, with 0.92, compared to an estimate of 0.79 in 2019.

Capture probabilities for females in aggregation 1 ranged from 0.22 to 0.30 and were consistent across sample days. For males it ranged between 0.09 and 0.72 and in 2018 was time dependent. For aggregation 2, female capture probability ranged between 0.25 and 0.55 and for males ranged between 0.21 and 0.79.

Table 2: Gross mark-recapture population estimates for each year and sex calculated from MARK using the models described in Appendix C3 with the sex ratio for that year calculated from the number of caught individuals. Total number of individuals caught for each year and sex are shown. There are no male estimates for 2017 as data collection began after their flight period

Aggregation	Year	Sex	Total caught	Total recaptures	Gross population	Standard Error	Change between years (%)	Sex Ratio (M:F)
1	2017	F	53	14	57.8	10.37		-
		F	116	19	379.5	81.5	147%	1:1.0
	2018	M	121	16	280.2	57.8	-	
		F	263	82	440.2	45.0	15%	1:3.4
	2019	M	78	22	91.6	12	-101%	
2	2017	F	23	7	24.5	5.7		-
		F	106	34	145.7	20.2	142%	1:2.4
	2018	M	43	12	54.8	13.1	-	
		F	107	32	182.6	28.4	23%	
	2019	M	25	7	37.9	24.4	-37%	1:4.3

The biggest change in population size were large increases from 2017 and 2018 for both aggregations (aggregation 1 147%, aggregation 2 142%). However, captures started much later in the flight season in 2017 compared to other years (Fig 4). For both aggregations the male population decreased between 2018 and 2019 (aggregation 1: -101%, aggregation 2: -37%).

For aggregation 1, the sex ratio based on caught individuals did not differ from an expected 1:1 in 2018 (exact binomial test, P=0.79, 1:0.96). However, was female biased in 2019 (exact binomial test, P<0.001, 1:3.4). For aggregation 2 sex ratios were female biased in both 2018 and 2019 (2018; exact binomial test, P<0.001, 1:2.4, 2019; exact binomial test, P<0.001 1:4.3, Table 2).

For both aggregations and all 3 years females reached their peak population estimate in mid - June (Figure 4). For aggregation 1 the highest daily population estimate was in 2019 (122.4±8.7, 9 June) versus 2018 for aggregation 2 (49.3± 5.23, 30 May Fig 4). Males emerged earlier than females in 2018 and 2019 and at both aggregations (Fig 4). For both aggregations male population peaked in late May and reduced throughout the survey period.

Both daily population estimates were higher in 2018 than in 2019 (aggregation 1: 99.9±31.1 vs 21.5 ±8.4, aggregation 2 :19.1 ± 6.4 vs 8.2 ± 3.5).

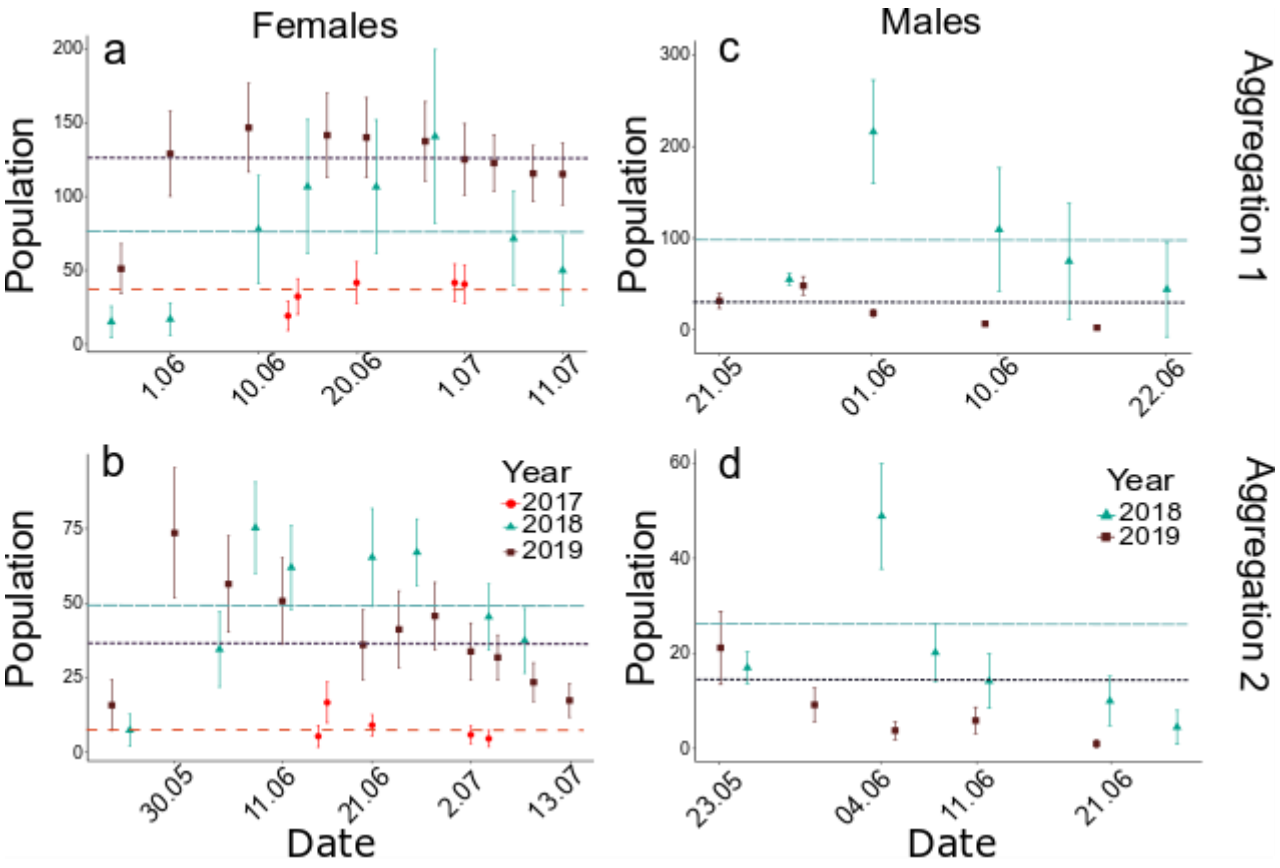


Fig 4: Daily population estimates for females for all three years and males for 2018 and 2019 at both aggregations a: females aggregation 1, b: females aggregation 2, c: males aggregation 1, d: males aggregation 2. Lines indicate the average daily population estimate and standard error bars are shown.

3.4.2 Floral availability

Floral availability was calculated from the mean percentage of inflorescences for each plant species or taxa present throughout the whole survey period. The mean percentage of each plant taxon was similar between aggregations (Fig 5) but with Fabaceae species more abundant surrounding aggregation 1. The most common plant species identified in surveys in aggregation 1 was *Trifolium repens* with an average of 195±39.1 inflorescences per 100m and an average of 18.0%±5.25% of the total inflorescences surveyed. *Vicia* species and *Lathyrus pratensis* (meadow vetch) had a combined average of 90.7 ±28.2 inflorescences per 100m. When grouped, all Fabaceae species (including *Lotus corniculatus* and *Trifolium*

species) on average accounted for 44.3%±7.20% of the inflorescences surveyed. A total of 39 non-Fabaceae species in bloom were identified in transects surrounding aggregation 1.

In aggregation 2, *Vicia* species and *L. pratensis* had a combined average of 145 ±59.4 inflorescences per 100m. All Fabaceae species comprised 13.1%±12.3% of the surveyed inflorescences. The most common plant species identified in surveys in aggregation 2 was also *T. repens*, with 144±19.2 inflorescences per 100m. A total of 47 non -Fabaceae species were identified surrounding aggregation 2.

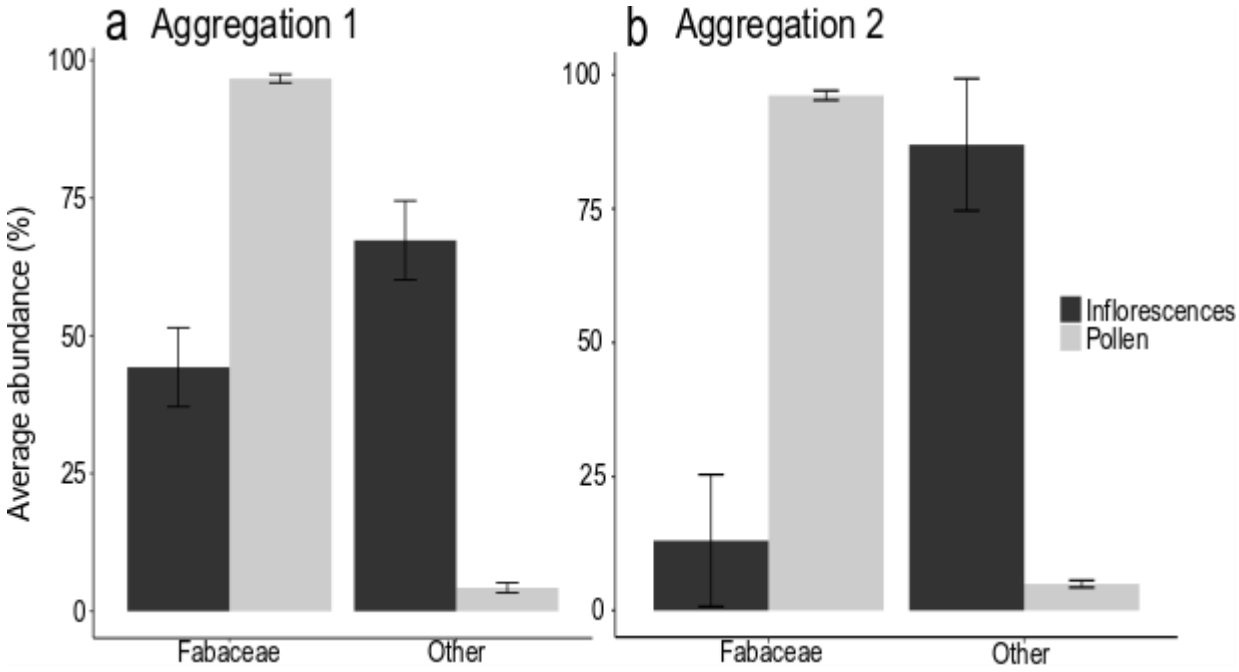


Fig 5: Relative flower abundance and pollen collected. Mean percentages of inflorescences present over the whole survey period for the Fabaceae, the most common plant group identified in pollen, and all other species present in the survey area. Also shown are the average percentage of pollen from samples collected from females in 2018. a: aggregation 1. b: aggregation 2. Standard error bars are shown.

3.4.3 Pollen samples

Across the three sample years a total of 113 pollen samples were collected from female bees at aggregation 1 and 64 from aggregation 2. Fabaceae pollen was present in 100% of samples from both aggregations (Table 3). This included pollen from various *Vicia*, *Lathyrus*, *Lotus* and *Trifolium* species. Fabaceae pollen made up over 90% of the pollen grains in every

sample for both aggregations (aggregation 1; 95.5±0.75%. aggregation 2; 94.6±0.89%, Fig 5). The remaining pollen grains were from seven species (Table 3) with bramble, *R. fruticosus,* the most common non- Fabaceae species (aggregation 1; 4.36 ±0.98%, aggregation 2; 5.44±0.66%). Fabaceae pollen was present significantly more than other plant taxa in pollen samples from both aggregations (aggregation 1: general linear model, $F_{1,230}$=2137, P<0.0001; aggregation 2: $F_{1,132}$=4370, P<0.0001). For aggregation 1 all samples contained pollen from more than one species. With aggregation 2, 97% of samples contained pollen from more than one plant group. Two different unknown plant species were identified in samples from 2017 and 2019.

Pollen composition was similar between years. However, for aggregation 1 in 2019 pollen from seven plant groups not previously identified in samples was present (Table 3). For a breakdown of daily pollen proportions from samples see Appendix C5 and C6.

Table 3: Plant groups identified in pollen samples from female *E. longicornis* for both aggregations for all years. How many samples each group was present in are shown. - indicates the plant group was not present in any samples that year. * indicates that when present the species accounted for less than 1% of the grains in a sample, potentially indicating contamination.

	Aggregation 1			**Aggregation 2**		
	2017	2018	2019	2017	2018	2019
Plant Group	Present on n/19 bees (%)	Present on n/25 bees (%)	Present on n/69 bees (%)	Present on n/14 bees (%)	Present on n/9 bees (%)	Present on n/45 bees (%)
Fabaceae	19/19 (100)	25/25 (100)	69/69 (100)	14/14 (100)	9/9 (100)	45/45 (100)
Trifolium repens	19/19 (100)	24/25 (96)	69/69 (100)	14/14 (100)	8/9 (89)	34/45 (76)
Lotus corniculatus	19/19 (100)	16/25 (64)	52/69 (75)	11/14 (79)	1/9 (11)	26/45 (58)
Rubus futicosus	13/19 (68)	15/25 (60)	1/69 (1)	-	7/9 (78)	5/45 (11)
Phacelia tanacetifolia	3/19 (16)	3/25 (12)	1/69 (1)	2/14 (14)	-	-
Asteraceae	1/19 (5)*	-	2/69 (3)*	-	-	-
Unknown	1/19 (5)	-	1/69 (1)	-	-	7/45 (16)
Clematis vitalba	-	-	3/69 (4)	-	-	1/45 (2)*
Trifolium pratense	-	-	5/69 (7)	-	-	1/45 (2)*
Silene dioicia	-	-	2/69 (3)	-	-	
Thypha latifola	-	-	1/69(1)*	-	-	

Filipendula ulmaria	-	-	1/69 (1)	-	-

3.4.4 Landscape composition

Fabaceae species were most common in meadow habitat, with *Vicia* species and *L. pratensis* together averaging 208 ± 35.9 inflorescences per 100m compared to 0 in woodland and managed grassland surrounding aggregation 1. For aggregation 2 the average per 100m was double, with 411 ±97.5 in meadow surveys and low but not zero in woodland 0.13 ±28.2 and managed grassland 0.88 ±110.

The landscape within 100m was 73% meadow and 27% woodland for aggregation 1 and 28% meadow, 55% woodland and 17% man made (a carpark) for aggregation 2. Within 500m of both aggregations, managed grassland had the greatest area (aggregation 1:36.8%, aggregation 2:34.2%, see Appendix C7).

During the floral surveys one unmarked female and male were caught. The female was caught approximately 300m to the South West of aggregation 2 (51.158382, -0.199089) within the meadow habitat. The male was caught approximately 700m to the North East of aggregation 2 (51.161666, -0.189562), also in the meadow habitat. The female was foraging on *L. pratensis* and the male on *T. repens.*

3.5 Discussion

E. longicornis at Gatwick airport is an example of how human intervention and land management can aid wildlife conservation of a rare and endangered species. The two nest aggregations are both in man-made habitats and appear to have stable female populations of reasonable size, so far as this three-year study can reveal. Aggregation 1, the larger, had estimated female populations of over 400 in 2019 and 380 in 2018, versus 146 and 183 for aggregation 2. Fabaceae species were the main pollen sources for *E. longicornis*, averaging 95% of samples. Within 100m of the nest aggregations these Fabaceae species were abundant and many were also growing in man-made or altered habitats.

3.5.1 Population estimates

Estimates appear to indicate that the populations were approximately stable between 2018 and 2019 with both populations smaller in 2017. However, surveys in 2017 were for a much

shorter period and did not include male estimates due to starting after the period of male activity. Males emerged before females, with peak population estimates either in late May or early June, compared to middle to late June for females. Earlier emergence of males is common in solitary bees and other insects (Wiklund & Fagerstrom 1977) and is generally due to mating strategy, with males waiting to mate with newly emerged females.

Both aggregations had female biased sex ratios in 2019, (aggregation 1, 2019 1:3.4; aggregation 2, 2019 1:4.3). In solitary bees, sex ratios have been found to deviate from the predicted Fisherian 1:1 ratio (Rosenhiem et al 1996). These deviations are often due to differences in parental investment for each sex as well as local mate competition and limited egg production (Herre 1985, Rosenhiem et al 1996). Sexual dimorphism is common in Hymenoptera, generally with females being larger (Helms 1994). This is true for *E. longicornis,* with males on average weighing 0.048g compared to a female average of 0.066g (Saunders pers. comms.). This results in an expected male bias sex ratio in the population, as males should be less costly to produce. However, when local resource availability is good this can result in investment in the more costly sex to be advantageous (Kim 1999). This is because females can allocate more resources to each individual offspring (Peterson et al 2006). Perhaps the increase of both female populations indicates favourable foraging conditions in the areas surrounding the aggregations.

We are unsure as to why the aggregations in this study have such a high female sex bias, and why this bias was not present in aggregation 1 in 2018. Although local mate competition and inbreeding can cause female bias (Herre 1985), this seems unlikely to be the case with *E. longicornis,* as mating occurs outside the natal nest and males are seen searching for mates at the nest sites. From a conservation perspective, female bias may be advantageous. The males present would be sufficient to fertilise the females and the larger number of females would boost the number of brood reared, with the effect on effective population size being small (Wright 1933).

In the Hymenoptera the risk with a highly skewed sex ratio in a population is the reduction of the equilibrium number of sex-alleles. However, the population size estimated for *E. longicornis* at Gatwick appear to be greater (>100) than would give cause for concern in terms of sex alleles and diploid male production in a species with complementary sex determination (Zayed 2004).

It is possible that the biased sex ratio is due to the methods used, with females perhaps easier to capture than males. However, capture probabilities for males were estimated to be higher than for females in 2019. Survival estimates for males in 2019 at both aggregations were lower than in 2018 and lower than the female estimates, with a large reduction in survival for males in aggregation 1 between years (2018;0.93, 2019;0.36). We are unsure as to why the estimated survival rate for males was so much lower in 2019 for aggregation 1. The difference in survival between males and females may be due to males not having a nest to return to either at night or during times of bad weather (Alves-dos-Santos et al 2009). Males also often disperse from the natal nest site (López-Uribe et al 2015) and if not caught before this time would not be included in the population estimate. Higher mortality or dispersal rate of males could partially explain the female bias sex ratio seen in these populations.

By placing emergence traps and digging up nests and examining cells the true sex and investment ratios could be determined.

3.5.2 Nest site characteristics

E. longicornis tends to nest on south facing, soft banks of bare soil or cliff face in the locations where it is found (Saunders 2014). In agreement with this, both Gatwick aggregations are nesting in sloped banks. Both these nesting sites exist as a result of human intervention. At Gatwick Airport, aggregation 1 is nesting on a manmade raised mound of excavated soil, and aggregation 2 along the bank of the river Mole (Fig 3). In 1999 Gatwick Airport diverted a section of the river to allow the expansion of the airport's western boundary. A new channel to the west of the old course, plus the shortening of a connecting western tributary, resulted in the river being moved a few hundred meters to the west of the original course. This new channel was altered to meander and allowed the creation of new habitats, such as the meadow along the length of the channel (British Airport Authority, 2004). Through the altering of the river, the bank with aggregation 2 was created. Some of the soil excavated during the project was left on Gatwick land to eventually become the heap in which aggregation 1 is located. It is unlikely that *E. longicornis* were present before this land management. This an example of how an industrial/commercial area, such as an airport, can manage the surrounding landscape in such a way as to create habitats and thereby help the conservation of a nationally rare species of wildlife.

Both nest aggregation sites have little vegetation cover and many patches of bare ground, a common characteristic of ground nesting bees (Wuellner 1999, Sardiñas & Kremen 2014).

Aggregation 1 has not moved from or expanded its nest location since it was first identified in 2014 (Rachel Bicker Pers comms). As of 2018, aggregation 2 has extended 20m further along the bank from its original location.

Surveys conducted along the banks of the river Mole and along suitable banks near aggregation 1 as part of this project as well as surveys by the Gatwick biodiversity team, have not discovered additional nest aggregations. Many species of solitary bees exhibit nest philopatry (Yanega 1990, Potts & Willmer 1997) in which newly- emerged females nest at their natal nesting site, instead of leaving to nest elsewhere. This will often have selective value for a foundress, as the natal nest site was successful enough to produce adults in the previous year, and therefore, is likely to be suitable again (Potts & Willmer 1997).

Limited availability of nest sites is also a reason why females may nest in their natal area instead of finding a new site (Batra 1978). However, at Gatwick there appear to be many banks of soft, bare soil, like the ones which already contain aggregations close by at both aggregations (<10m). If the female population continues to grow and nest density capacity is reached, dispersal from these aggregations is possible with new aggregations potentially being formed beyond the area surveyed. A male *E. longicornis* was sighted approximately 1km from aggregation 2 (Pers comms. Laurie Wright). This male may be from one of the aggregations in this study, or potentially a new aggregation not identified. Future surveys covering a wider area are required.

3.5.3 Pollen composition

Pollen collected from returning females from both aggregations was predominantly Fabaceae species which was present in all (113) samples taken from individual females and always accounted for more than 90% of the total grains in a sample. Pollen was identified as *Trifolium, Lotus, Lathyrus* and *Vicia* species. The most common non Fabaceae species was bramble, *R. fruticosus* which averaged 5%. This narrow familial range of pollen collection indicates that *E. longicornis* is oligolectic (Muller & Kuhlmann 2008) and agrees with previous research (Saunders 2014). Visual sightings of males on *Symphytum orientale* (white comfrey), a species of pollen not identified in female pollen loads, indicates a slightly broader foraging range than for female pollen collection. Differences in male and female foraging preferences are often seen in solitary bee species (Ritchie et al 2016) even in those with a narrow diet breadth. This is most likely due to males not needing to collect pollen for cell provisioning. In addition, male solitary bees normally emerge earlier than females, as seen in

this study, so that the floral composition in the area will be slightly different, influencing what species they forage on.

3.5.4 Landscape composition

Fabaceae pollen sources were most abundant in the meadow habitats for both aggregations, and within 100m of aggregation 1 meadow is the predominant habitat type (72%). The proximity of the appropriate floral resources to the nesting sites is important as *E. longicornis* is reported to have a foraging range of approximately 500m (Saunders 2014). An abundance of good quality forage near to the nesting site helps reduce the costs of foraging and therefore potentially increases reproductive output (Zurbuchen et al 2010).

3.5.5 E. longicornis conservation status

E. longicornis is in decline in Britain and other parts of its range (Pekkarinen 1997, BWARS 2012). In Cornwall, a stronghold for the species, there has been a 55% reduction since 1987 in the number of sites where it is found, from 24 to 11 (Saunders 2014). This is thought to be due to a combination of the loss of flowering legumes, increased extreme storm events along their coastal sites destroying nests, and a lack of sites containing both early and late flowering legume species such as *Anthyllis vulneraria* and *Lathyrus sylvestris* (Saunders 2017). Fabaceae species often occur in flower rich grasslands, which are declining in the UK (Goulson et al 2005). This is due to a variety of factors, predominantly changes in agricultural practices. Between early and late 20th century, fodder crops declined by more than 55% and were replaced with permanent grassland (Ollerton et al 2014). These changes in landscape management have resulted in both a reduction in the range of many of the pollen sources *E. longicornis* rely on, as well as their frequency at a more local scale. For example, *L. pratensis,* a major pollen source for *E. longicornis,* reduced in frequency by 45% in the UK between 1978 and 1998 (Carvell et al 2006). Owing to airports often having semi-natural grasslands surrounding runways, they have the potential to provide this much declining habitat which contains the floral resources that *E. longicornis* and other species need.

3.5.6 Management plans and future research

Although not known for their wildlife value or biodiversity, if managed correctly airports can provide a refuge for some species, as seen with *E. longicornis* at Gatwick. Another example is the presence of rare ground nesting birds at John F. Kennedy (JFK) airport in New York

City, USA. The upland sand piper, grasshopper sparrow, and horned lark have all been found nesting in the airports semi-natural grassland habitat (Chevalier & Cohen 1997, Kershner and Bollinger 1996). A high abundance and diversity of arthropod species have also been identified at JFK (Kutschbach-Brohl et al 2010), highlighting that if managed correctly airports have the potential to benefit wildlife.

There are over 60 major airports in the UK (Civil Aviation Authority 2020) and with the growing demand for airline travel the size and demand on airports is likely to increase (Upham et al 2003). This highlights the importance of surveying airport land for wildlife and ensuring the land is managed effectively. Currently, Gatwick Airport's management of the study area involves a single September cut of the meadow around the two aggregations, and coppicing of the woodland to help create and maintain compartments for wildflowers (Rachel Bicker Pers comms.), which should be continued. Ensuring the woodland surrounding the aggregations does not begin to encroach on the meadowland is also essential (Table 4).

Continued monitoring of the aggregations is recommended and, hopefully, to confirm that the existing management is effective. The mark recapture method used in this study is highly intensive and therefore, is likely to be impractical for future monitoring of population sizes unless additional staff are recruited. However, the population can be monitored in other ways. Counts along transect routes, recording number of nest holes at each aggregation site, and fixed standardised counts of activity at the aggregations are all potential population monitoring methods. For further information on management and monitoring suggestions see Table 4. These latter methods require less training but can still give a quantitative measure of population size (Bischoff 2003, Larsson & Franzén 2008) that could be compared across years to quantify any trends or changes. From what we can see from the current management and situation, it appears the two aggregations at Gatwick Airport are not threatened. Both have additional unused nesting space nearby and abundant forage and both foraging and nesting location are not in danger of becoming unsuitable either from natural or human factors.

Future work on these aggregations could consist of surveys of the surrounding soil banks, which are currently not being used as nesting sites, to determine if new nesting aggregations are founded. *E. longicornis* is also a key candidate for citizen science due to the males distinctive and unusual appearance, with their long antennae unlike any other native bees to the UK. This would make is possible to carry out a large-scale survey of the surrounding area

using information from local residents. In 2018 a male was recorded approximately 3km South East of the existing aggregations (NBN atlas 2018), perhaps indicating more nesting sites in the local area.

In many cases human changes to the landscape are detrimental to species and biodiversity (Winfree et al 2011, Senapathi et al 2015b). However, through effective management biodiversity can be maintained and some species can even thrive in these human-altered landscapes (Macadam & Bairner 2012, Sirohi et al 2015). Although *E. longicornis* is in decline throughout its range, by monitoring and conserving small populations like those at Gatwick, the conservation of this charismatic species may be possible.

Table 4: Land management and monitoring suggestions to ensure the continued conservation of the two aggregations of *E. longicornis* on Gatwick land. The suggestion along with the desired outcome, how often it should occur, and the number and specialisation of individuals needed are shown

Habitat Management Suggestions			
Suggestion	**Outcome**	**Occurrence**	**Individuals required**
End of summer mowing of meadow	Allows for continued growth of important meadow species (e.g. Fabaceae) for *E. longicornis*	Once a year at the end of September	-
Coppicing of woodland and scrub	Creates compartments for wildflowers to grow within woodland. Prevents woodland and scrub encroachment onto the adjacent wildflower meadow	Coppicing of the woodland edge approximately every three to five years and inner woodland every 5-20 years (Royal Forestry society)	-
Clearing of vegetation on existing and potential nesting aggregations	Maintains the short grass and bare soil preferred by *E. longicornis* for nesting and helps maintain potential nesting sites.	Once a year at the end of September	-
Monitoring Suggestions			
Transect walks in surrounding meadow, recording the number of male and female *E. longicornis* seen	To determine a population estimate for both aggregations combined	Once a week for the length of the species flight season (Mid May until end of July)	One/two individuals who are trained and able to correctly identify *E. longicornis*
Counting individuals at nesting aggregations	Calculate a population estimate for each aggregation separately	Once a week at each aggregation for a fixed time period (~1-2 hours) for the length of the species flight season	One/two individuals who are trained and able to correctly identify *E. longicornis*
Counting number of nests at each aggregation	Calculate an estimate of the number of females at each nesting aggregation	Two/three times a week at each aggregation to get a weekly average for length of the species flight season. Would take approximately 10-15 minutes each visit	One individual who is trained and able to identify *E. longicornis* and their nest entrances. N.B. Some females share nest entrances (Westrich 1989) so this is a less accurate method
Surveys of surrounding banks for other nesting aggregations	Identify if there are any more nesting aggregations near existing nest sites	Once/twice during the flight season conduct slow transect walks along banks adjacent to aggregations to identify nesting females or patrolling males.	Two/three trained individuals, or even recruit local naturalists

Chapter Four: Phenology of the specialist bee *Colletes hederae* and its dependence on *Hedera helix* L. in comparison to a generalist, *Apis mellifera.*

4.1 Abstract

Colletes hederae, the ivy bee, (Hymenoptera: Colletidae) has undergone large range expansions in Europe in recent years, including colonising Britain in 2001 with its original distribution limited to Western mainland Europe and the Channel Islands. It is thought to specialise on *Hedera helix* L. (Apiales: Ariliaceae), common ivy. However, some research has questioned this dependence. This study quantifies the foraging ecology of *C. hederae* to determine its relationship with ivy in Sussex. We quantified the phenology of ivy bloom, *C. hederae* activity and flower visitation, and pollen collection of females through pollen analysis. We also gathered equivalent data on *Apis mellifera* both as a comparison and to assess alternative pollen sources. The phenology of female *C. hederae* activity was highly correlated with and phonologically contained within the ivy bloom period. Pollen analysis from *C. hederae* identified ivy pollen was 98.5% of samples, significantly more than for *A. mellifera* (90%). Two other plant species were identified and more common in *C. hederae* samples when ivy bloom was not at its peak. Surveys of ivy flowers surrounding the aggregations found that *C. hederae* were the most common insect (26%) foraging on ivy. Although *C. hederae* can forage on other species, ivy was found to be an important floral resource. The results also suggest the potential for competition with *A. mellifera*, the only other bee present in more than small numbers on *H. helix* flowers, as although *A. mellifera* is a generalist, in autumn it mainly forages on ivy.

4.2 Introduction

When a species specialises on a single food source it has a total dependence on the distribution of this resource across time and space. Herbivores can be specialists or generalists in their use of food plants. Specialisation is seen between many insect species and their host plants (Kéry et al 2001). For example, *Argynis adippe* (the high brown fritillary) whose larval food plant is predominantly *Viola riviniana* (common dog violet, Barnett & Warren 1995) and *Pieris rapae* (small white butterfly) whose larvae primarily consume plant species from the cabbage family (Davies & Gilbert 1985). However, this level of specialisation is rare between adult insect pollinators and their host flowers (Minckley & Roulston 2006).

Unlike other pollinator species such as butterflies and flies which only forage on pollen and nectar when in the adult stage, bees also rely on these to feed their larvae (Nicolson 2011). Nectar is predominantly an energy source, with pollen providing bees with protein, lipids, and minerals which are essential for larval development (Vanderplanck et al 2017).

Host-pollen specialisation in bees is categorised in three levels (i) polylecty, where bees collect pollen from a broad range of flowers, e.g. *Apis mellifera* (Köppler et al 2007), (ii) oligolecty, where pollen is collected from a narrow range of species within a genus or related genera e.g. *Colletes halophilus* (Sommeijer et al 2009) and (iii) monolecty, where bees collect pollen from a single plant species e.g. *Hesperapis oraria* (Davis et al 2020, Müller & Kuhlmann 2008).

The floral composition of a landscape is usually heterogenous both spatially and temporally, potentially posing a challenge for bees which are reliant on specific floral hosts. Species which do demonstrate a narrow foraging range will have matched phenology with their food plants (Minckley et al 1994, González-Varo et al 2016, Ogilvie & Forrest 2017) often with emergence of females occurring slightly before the peak floral bloom (Larsson & Franzén 2008). Cases of complete mismatch between floral host and oligolectic bee species are rare. However, sometimes emergence can be slightly out of synchrony with the floral bloom. Here, specialists have been seen to adapt and forage on other available species (Westrich 2008, Müller & Kuhlmann 2008) resulting in a suggested reclassification of the three pollen specialist categories to account for the flexibility often seen in species (Cane & Sipes 2006, Müller & Kuhlmann 2008, Cane 2020).

Colletes hederae, (Hymenoptera: Colletidae), the ivy bee has previously been suggested as being narrowly oligolectic on pollen from *Hedera helix* L., common ivy (Apiales: Ariliaceae), or under one of the proposed changes to the classification system, monolectic within the *Hedera* genus (Cane 2020). However, studies both on museum specimens (Müller & Kuhlmann 2008) and live bees (Teppner & Brosch 2015) in Europe have suggested it may have a wider diet range. Pollen specialisation is also seen in the closely related *C. succinctus* and *C. halophilus* species, which specialise on heather species and sea aster respectively (BWARS 2011, BWARS 2012).

Colletes hederae was only described as a new species in 1993. At that time it was known only in the Channel Islands, France, Germany, Italy and Croatia (Schmidt and Westrich 1993). Since then its range has spread and it is now found throughout southern and western Europe (Bischoff et al 2005) with its range much smaller than that of its host plant species, *H. helix.* Since its arrival in southern England in 2001, *C. hederae* has spread throughout Britain. It is now recorded in multiple sites in the southern half of Britain and as far north as the Yorkshire and Lancashire coasts, 400 km from the first record in Dorset (BWARS, 2019).

This expansion of an invasive species could potentially place pressure on the native pollinator community. This is especially the case for *C. hederae* as they are active in early autumn and forage on the main source of nectar and pollen for most pollinator species active at this time. There is growing evidence that *Apis mellifera* (Hymenoptera: Apidae) can compete with wild bee species for floral resources (Hudewenz and Klein 2015, Herbertsson et al 2016) in part due to them being generalist foragers (Köppler et al 2007) and living in large colonies. Their diverse diet, and the fact that *A. mellifera* are known to forage heavily on *H. helix* during the autumn (Garbuzov and Ratnieks 2014) mean they make for an ideal comparison between the specialist *C. hederae*, and can help give an indication of the floral resources available in the surrounding area as they are able to forage across large distances (Couvillon et al 2014). Also, by comparing these two species' activities on *H. helix*, it may help identify the potential competition posed by *C. hederae* on *A. mellifera* and other *H. helix* visiting insects.

Colletes hederae are known to have been present on the University of Sussex campus since at least 2009 (Garbuzov and Ratnieks 2014). The aim of this investigation was to quantify the foraging ecology of *C. hederae* and to determine its relationship with *H. helix* (hereafter referred to as ivy) in East Sussex, an area of the UK where it has been present for approximately 10 years and is now abundant. This was achieved through (i) phenology,

comparing *C. hederae* nesting and foraging activity with the local ivy bloom intensity, (ii) pollen analysis using samples collected from foraging females returning to their nests in comparison with samples from *Apis mellifera* hives (iii) surveys of insects visiting ivy flowers.

4.3 Methods

4.3.1 Study Species

4.3.1.1 Colletes hederae

Colletes hederae is a medium-sized solitary bee, with females up to 13mm long and males up to 10mm (Falk and Lewington 2015). Female ivy bees are very similar to honey bee workers in size. Adults are mainly on the wing from late August to mid-October, coinciding with the bloom of its host plant, ivy (BWARS). *Colletes hederae* females nest in densely populated aggregations in the ground, with reports of up to 300 nests per m^2 (Bischoff et al 2005). A single female can produce 18 brood cells in one month of adult activity (Danforth et al 2019). Females will collect both nectar and pollen for their brood.

4.3.1.2 Apis mellifera

In comparison to *C. hederae*, *Apis mellifera* are eusocial and live in large colonies with many thousands of individuals (Seeley 2009). They forage all year round, provided floral resources are available, and the temperature is above 10°C (Seeley 1989). They collect both pollen and nectar from a variety of flower species (Köppler et al 2007).

4.3.1.3 Ivy

In Britain there are two species of ivy, *H. helix* and its tetraploid daughter species *H. hibernica*. Both are common and widespread throughout the UK, except for some parts of Northern Scotland, and are almost identical in morphology (McAllister 1990). Due to the difficulties in identification, the two species were grouped and classed as ivy. Ivy generally grows in shaded, damp locations, and is often found in woodland (Metcalfe 2005). It only begins to flower once mature, aged 10 years or more, where the leaves also change from being lobed to ovate (Clark 1983). Ivy flowers are pentapetalous and contain an open type nectary, which is easily accessible to pollinators (Konarska 2014). Flowers occur in panicles of 1-6 umbels, with anthesis occurring 1-2 days before nectar secretion (Metcalfe 2005).

4.3.2 Nest Site Aggregations

Three nest aggregations of *C. hederae* were studied in September and October 2019. One was in the garden of a house in the village of Falmer, East Sussex, approximately 300m from the edge of the University of Sussex Campus (50.864189, -0.079073). The second was on the University of Sussex campus (50.866395, -0.089911) on a small (c. 115m^2) grassy slope and the third was located on the edge of the town of Lewes, approximately 5km to the East of Falmer (50.880111, -0.000481). The Lewes aggregation consisted of two small aggregations (c. 23m^2 each) which were present on the front lawn of neighbouring houses. Due to their close proximity, 20m apart, they were combined for analysis.

4.3.3 Pollen collection and analysis

4.3.3.1 Colletes hederae

Pollen was collected at all 3 aggregations from foraging females with visible pollen loads returning to their nests. Once caught, a bee was transferred to a honey bee queen marking cage (Pixnor, China) where using a toothpick pollen was scraped from both hind tibia and placed in individual Eppendorf tubes and stored for later analysis. Once pollen was collected, bees were released. Between 10-20 samples were collected per week per aggregation. Sampling started when females were first returning to their nests with pollen loads until they were no longer active at the nesting sites. For the Falmer aggregation this was from 16 September to 21 October and for the Sussex University (SU Campus) and Lewes aggregations this was from 16 September to 7 October.

For pollen identification, each sample was then placed on a microscope slide and mixed with a small volume of glycerine jelly stained with Fuchsin dye. A cover slip with a small grid was used allowing the sample to be divided into equally sized quadrats. The section of sample to be analysed was chosen at random and the number of pollen grains per plant species within each section was counted. Once all the grains within a section were counted, a new quadrat would be chosen at random and the process completed until 200 grains had been counted. If there were not 200 grains in a sample, the entire sample was analysed. Pollen identification and counting occurred using a compound microscope at 400x magnification using both a pollen reference collection from flowers surrounding the aggregations and a pollen identification guide (Sawyer and Pickard 1981). For each sample, the proportion of each species present was calculated. Classification to species level was attempted. However, due to similarities between species this was not always possible e.g. Asteraceae species.

4.3.3.2 Apis mellifera

Pollen was also collected from four *Apis mellifera* colonies in hives at the Onion Field apiary, c. 40m from the Falmer aggregation of *C. hederae*. To collect pollen from *A. mellifera* hives, a standard pollen trap with a 5mm plastic mesh, (E.H Thorne UK) was attached over the entrance of each hive. This mesh results in pollen from returning foragers being knocked from the pollen baskets into a tray. Mesh was put into position one day per week from 8:30 to 17:30 to encompass an entire foraging day on days of good weather for bee flight (above 10°C and no rain). Each sample comprised the pellets collected from one hive on one day. The survey period was 26 August to 28 October with no pollen collected on the week of 21 October as there was no suitable foraging weather.

In most cases the daily sample from a hive was large so a sub sample of 50 randomly chosen pellets was used. Occasionally (2/25) the sample was less than 50 pellets, and all were identified. Occasionally hives returned no pellets (8). Each pellet was of a single colour indicating that the pollen was collected from a single plant species. This is expected as *A. mellifera* are flower constant, with almost all foragers visiting only one species of plant per foraging trip (Free 1963).

Pellets were separated by colour and if a colour had more than 10 pellets, then 10 were randomly chosen and analysed. If all 10 were the same species, the remaining pellets of that colour were tallied together and not examined under the microscope. A small amount from each pellet was mounted onto a slide, as for *C. hederae*, mixed with glycerine jelly stained with Fushin dye and examined at 400x under a light microscope using the same quadrat system as with *C. hederae*. Pollen was again identified to species where possible using the same pollen identification guide as with *C. hederae* (Sawyer and Pickard 1981).

4.3.3 Ivy Bloom Period

To determine the bloom period of ivy all patches of ivy present within 300m of each aggregation were surveyed and the bloom quantified. Foraging range of solitary bees is thought to correlate with intertegular (IT) span and body length, which for *C. hederae* (IT span 1.5-2mm, body length 13mm Female, GH Pers obs) indicates a foraging distance of 500m (Gathmann & Tscharntke 2002, Greenleaf et al 2007). Due to the high local abundance of ivy and because bee foraging distances reduce with increased floral resources (Gathmann et al 1994 , Gathmann & Tscharntke 2002), 300m was chosen as a practical and ecologically

relevant survey distance which would give a good indication of the local ivy flower availability surrounding the aggregations.

Once per week from 9 September to 21 October 2019 fixed survey routes within 300m of each aggregation were walked. Mature ivy is often found in abundance along walls, hedges, and trees. In order to survey the ivy present was allocated to patches 2m long and 2m high and each of these patches was given a score to indicate the proportion of flowers in bloom. For practical reasons we had to limit the height surveyed to 2m even though some ivy was higher. This ensured that flower assessment was accurate as above 2m it was not possible to accurately determine the bloom stage of flowers. Each patch was given a score to determine its flowering status: 0) buds only, with no flowers open or all flowers have finished blooming; 1) 0-30% of flowers open; 2) 30-80% of flowers open; 3) Peak bloom, 80-100% of flowers open (Fig 1). This classification was adapted from Garbuzov & Ratnieks 2014. For analysis, the midpoint percentage of flowers open for each patch was determined (e.g. a score of 1 indicates 15% of flowers open). The average percentage of open flowers for each survey week was calculated as the average of all flower patches for each site.

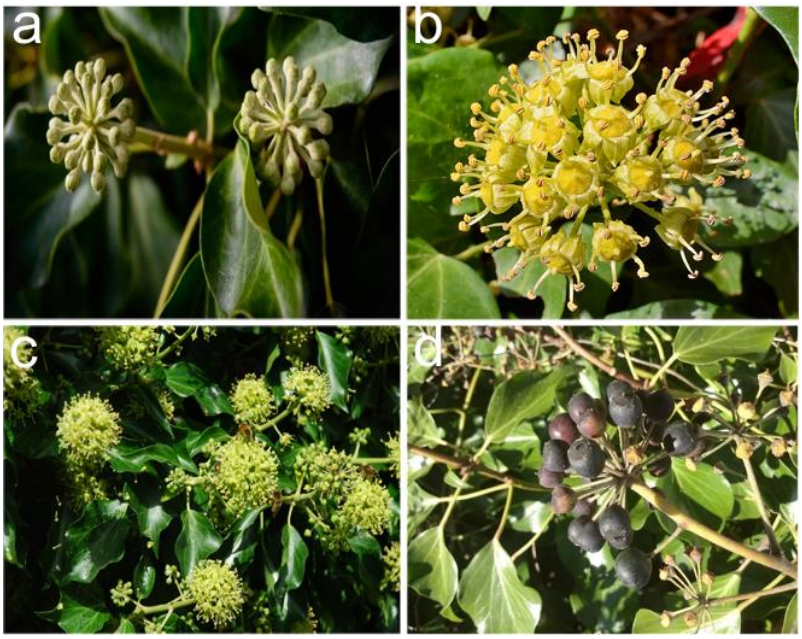


Fig 1: Ivy flowers at different stages of bloom. a) Flowers have not yet opened so would be classed as a 0 (Christina Pictures 2016), b) Open flowers. Depending on the number open within a patch a score ranging from 1-3 would be given (Nick Upton 2018), c) A patch of flowers which would be classified as a 3, full bloom, as more than 80% of flowers are open (Alan Fryer 2007), d) Flowers have 'gone over' and berries have formed, a score of 0 (Linda Crampton 2017)

4.3.4 Colletes hederae activity at nest aggregation

Activity was quantified by the number of *C. hederae* individuals on the wing at each aggregation. This was determined by sweeping with the same insect net at each aggregation and calculating the average number of male and female *C. hederae* caught per sweep. Sweeps were conducted on days with weather good enough for insect flight (sunny, minimum temperature 11°C between 10:00 and 16:00). Three sweeps of the entire aggregation were done each day with surveys attempted every day. However, due to weather conditions this was not always possible. Bee activity surveys occurred between 9 September until 21 October as this covers the main period when *C. hederae* are on the wing (Falk and Lewington 2015).

4.3.5 Insect surveys

Insects on ivy flowers were surveyed in the village of Falmer and on the University of Sussex Campus with identification by eye in the field. Surveys in Falmer were within 300m of the Falmer aggregation, with surveys on the University of Sussex Campus occurring between 500-650m away from the SU aggregation. Within these sites several distinct areas of ivy flowers were surveyed, with the specific patches used varying between weeks as areas came in and out of bloom. Surveys took place at approximately weekly intervals and corresponded with the main ivy bloom from 2 September to 21 October. Counts of insects on flowers were made until a total of 100 insects were identified at each of the two study areas. On some weeks fewer than 100 insects were counted on the Campus site (n=79, 23 September, n=50 14 October, n=86 21 October). On a few of the survey weeks data was collected over multiple days (5 occasions). Data were collected at times of weather good enough for insect flight (temperatures ranged from 10-21°C) between 10:00 and 16:00. All ivy flower visitors were identified to the following taxa: 1- *Colletes hederae*, 2- *Apis mellifera*, 3- *Bombus*, 4-Apoidea (other bees), 5-*Vespula vulgaris*, 6-Syrphidae (Hoverflies), 7- Diptera (other flies) and 8-Lepidoptera (butterflies). The number of male and female *C. hederae* was noted, as was the proportion of female *C. hederae* and *A. mellifera* with visible loads of pollen.

4.3.6 Statistical analysis

The relationship between female *C. hederae* activity at aggregations and ivy bloom intensity (percentage of open flowers) was analysed using a generalised mixed effect model (GLMM) with a negative binomial distribution and aggregation site as a random factor. The response variable was the number of females caught per survey. The predictor variables were percentage of open flowers and survey week. Week was included in the model as a quadratic function as this was found to best fit the data.

To test if the average proportion of ivy pollen across the entire survey period differed between *C. hederae* aggregations a Kruskall-Wallis test was run with a follow on post hoc Dunn test with Bonferroni correction. To determine if the proportion of ivy pollen present changed over time for each *C. hederae* aggregation, a Chi Square test for heterogeneity was run for each aggregation comparing ivy proportions for each survey date.

To determine if the proportion of ivy collected differed between *A. mellifera* and *C. hederae* a Wilcoxon Signed Rank test was performed with percentage of ivy as the dependent variable and bee species as the independent. Only pollen collected by *A. melifera* hives during the

same period that female *C. hederae* were on the wing was used in the comparison (9 September to 14 October). Pollen collected from *C. hederae* at the Lewes nest aggregation was not compared with *A. mellifera* because this aggregation was located beyond the foraging range of the *A. mellifera* hives. All graphs were made and tests run using R (R Core team, version 3.4.2).

4.4 Results

4.4.1 Colletes hederae pollen composition

A total of 203 pollen samples were collected from individual female *C. hederae*, 101, 40 and 62 from the nest aggregations at Falmer, Sussex University Campus (SU Campus) and Lewes, respectively. Ivy pollen was present in 100% of the samples and on average was 98.5 ± 0.29% SE of the pollen grains counted. In 65% (131/203) of the samples ivy was the only species identified. Ivy pollen proportions were high at all three locations. However, there was a significant difference between Falmer and Lewes (99.1± 0.22% SE vs 97.3 ±0.76% SE, Dunn post hoc test, χ^2= 10.40, df=3, P=0.002, Fig 3a). There was no difference between SU Campus (98.7±0.60% SE) and Falmer (P=0.84) and Lewes (P=0.70, Fig 2a).

There was no significant difference between the proportion of ivy pollen in samples across weeks for Falmer (χ^2 = 1.49, df=5, P=0.91). However, there was for SU Campus (χ^2 = 11.9, df=3, P=0.007) and Lewes (χ^2=15.79, df=3, P=0.001). Figure 3a shows that the lowest proportion of ivy in samples for both Lewes and SU Campus were from the week commencing 16 September (92% Lewes, 94% SU), at the start of the ivy bloom. After this the proportion of ivy pollen present per sample increased to over 98% at both.

Pollen from four other plant groups were identified (Table 1). Across aggregations, the second most abundant was wild clematis, *Clematis vitalba* (Ranunculales: Ranunculaceae), which was present in 21% of samples, 0.89 ± 3.39% SE on average per sample (Table 1). The other three groups identified included bramble, *Rubus fruticosus* L. (Rosales: Rosaceae), Asteraceae species and birds foot trefoil, *Lotus corniculatus* (Fabales: Fabaceae). All pollen species identified were from wild flower species. Asteraceae pollen was not observed in samples from the SU Campus aggregation and *L. corniculatus* was only identified in samples from Falmer. *Rubus fruticosus* and *C. vitalba* were found in samples from all three aggregations.

Clematis vitalba was also the second most common plant species in samples from Lewes (1.65 ± 0.72% per sample, present in 14/62 samples). On the week of 16 September, when ivy proportion was at its lowest for Lewes and SU Campus aggregations, the most common other pollen identified was *C. vitalba*. In Lewes it accounted for 8% and in SU Campus it accounted for 4% of the pollen identified that week.

4.4.2 Apis mellifera pollen composition

A total of 1187 pollen pellets were analysed from 25 sample occasions from 9 days. There was no significant difference in the proportion of ivy pollen among the four *A. mellifera hives* (Kruskall Wallis, df=3, P=0.538), all of which were located at Falmer, so they were pooled for analysis.

Ivy was present in 92% (23/25) of the *A. mellifera* samples and for the whole survey period (26 August to10 November) on average per sample was 67% of the pellets identified (Table 1). The second most abundant pollen type was *C. vitalba* (10.56 ± 3.27% SE of pellets, Table 1). When examining the presence of ivy pollen in samples only in the period that overlaps with *C. hederae* pollen foraging (16 September to 14 October) ivy was present in all samples and was 90% of pollen pellets identified. Throughout the whole survey period pollen from eight other plant groups were also identified (Table 1). *Rubus fruticosus* was present in more samples than *C. vitalba* but accounted for a smaller proportion of identified pellets (*R. fruticosus* 7.29± 2.48% SE vs *C. vitalba* 10.56± 3.27% SE). During the period which overlapped with *C. hederae* activity three other plant species were identified in *A. mellifera* samples, *C. vitalba, R. fruticosus* and a species which was not found in *C. hederae* samples, poppy *(Papaver spp.*, Table 1).

There was a significant difference in the proportion of ivy pollen in samples between weeks for *A. mellifera* colonies (χ^2=277.61, df=8, P<0.0001, Fig3b). The highest proportions were in the weeks commencing 7 October and 14 October when it was 100% of pellets. The lowest proportion was in the week of 26 August, with on average 25% of pellets being ivy (Fig 3b).

There was a significantly higher proportion of ivy pollen in *C. hederae* samples from 16 September to 14 October from the Falmer and SU aggregations compared to those of *A. mellifera* (Wilcoxon rank sum test, W= 663, P=0.044, 98.9± 0.23% vs 90.8±4.82, Fig 2b). Pollen from the Lewes aggregation was not compared with the *A. mellifera* colonies because it was located outside the foraging range of the *A. mellifera* pollen trap hives (Couvillon et al 2014).

Table 1: Plant species found in pollen samples from *Colletes hederae* and *Apis mellifera*. For *C. hederae* data are for all aggregations and for *A. mellifera* all hives, which were all at the Falmer location but also close to the SU campus aggregation. The number of samples where each species was present is shown together with the average proportion per sample. Species which were found in both *C. hederae* and *A. mellifera* samples are in bold. The asterisk, * indicates a potential contamination as was only present in one sample. Proportions for both the entire survey period and the period that overlaps with *C. hederae* pollen foraging are shown for *A. mellifera*.

***Colletes* hederae: 16 September – 21 October**			
Plant Species	Present in individual bee (*C. h)* or hive (*A. m*) per week (%)	Mean proportion across samples (%)	SE Standard Error (%)
Hedera sp.	100% (202/202)	98.5%	0.29%
**Lotus corniculatus*	0.5% (1/202)	0.009%	0.009%
Clematis vitalba	21.3% (43/202)	0.89%	3.39%
Rubus fruticosus	20.3 (41/202)	0.61%	3.39%
Asteraceae sp.	1.45% (3/202)	0.02%	0.007
***Apis mellifera*: 26 August- 28 October (whole period of ivy bloom)**			
Hedera sp.	92% (23/25)	67.10%	7.03%
Clematis vitalba	44% (11/25)	10.56%	3.27%
Rubus fruticosus	48% (12/25)	7.29%	2.48%
Papaver sp.	36% (9/25)	5.92%	2.27%
Dahlia sp.	16% (4/25)	1.61%	0.92%
Brassica napus	4% (1/25)	0.32%	0.32%
Phacelia tanacetifolia	4% (1/25)	0.24%	0.24%
Hypericium sp.	4% (1/25)	0.08%	0.08%
Epilobium	4% (1/25)	0.08%	0.08
Unknown	4% (1/25)	2.24%	2.24%
***Apis mellifera*: 16 September- 21 October (*C. hederae* foraging period)**			
Hedera sp.	100% (13/13)	90.80%	4.82%
Clematis vitalba	39% (5/13)	5.83%	3.50%
Rubus fruticosus	15% (2/13)	0.77%	0.53%
Papaver sp.	23% (3/13)	2.62%	1.75%

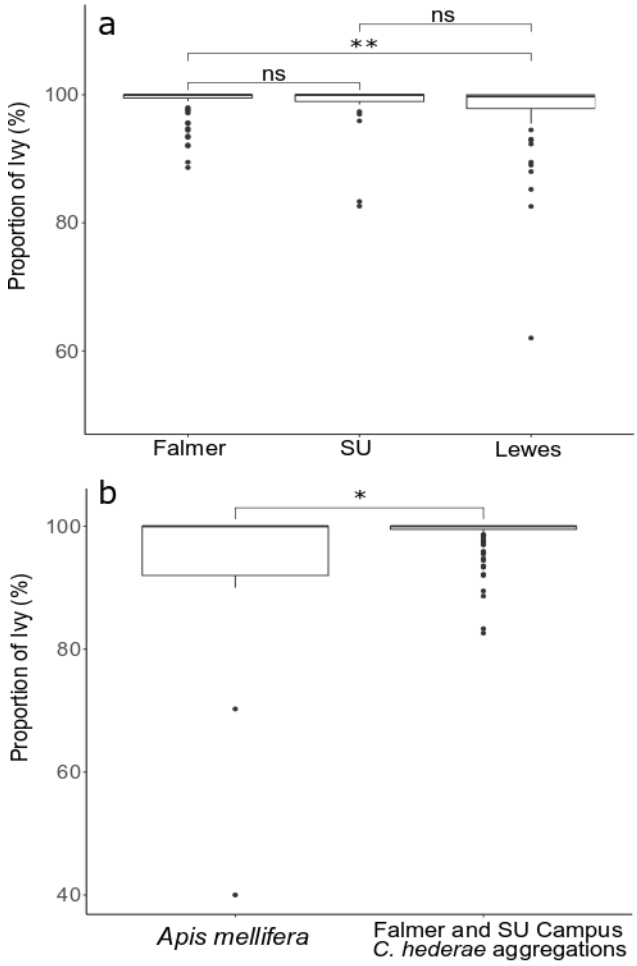


Fig 2: Proportion of ivy pollen in samples between 16 September to 21 October, the period *Colletes hederae* females were foraging for pollen in a) *C. hederae* samples for each aggregation and b) *Apis mellifera* colonies and the Falmer and SU Campus aggregations. Due to no difference in ivy proportion between Falmer and SU Campus these were combined for comparison with *A. mellifera*. Lewes was not compared with *A. mellifera* due to the aggregation being located beyond the foraging range of the *A. mellifera* hives. * indicates a significance of P <0.05 and ** indicates a significance of P<0.001. NS means no significant difference between the group was identified.

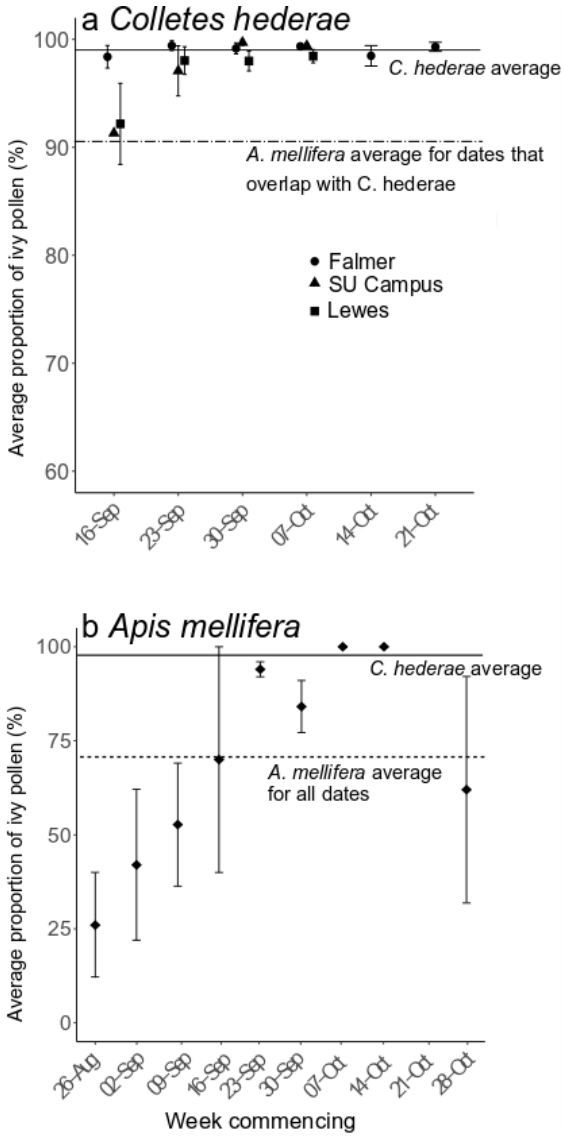


Fig 3: Mean proportions of ivy pollen present in samples for each survey week for a) *C. hederae* and b) *A. mellifera*. The dashed line shows the average proportion for dates that overlap with *C. hederae* flight for *A. mellifera*, the solid line shows the same for *C. hederae* and the dotted line shows the average proportion for the entire sample period for *A. mellifera* (26 August to 28 October). For *C. hederae* only samples from the Falmer aggregation were collected for the final two survey weeks due to low levels of activity at the other two aggregations (21-28 October)

4.4.3 Colletes hederae phenology

Falmer was the aggregation with the highest sweep counts, averaging 28 males and females combined versus 11.7 for Lewes and <1 for SU Campus. Due to the low numbers at SU Campus, it was not included in the phenology analysis. For both Falmer and Lewes aggregations male activity peaked before females (Fig 4), males were most active on the week of 9 September ($\bar{x}$=68.3 ±27.5 SE), and females on the week of 30 September ($\bar{x}$=17.9 ±1.98 SE). This peak in female activity corresponded with the peak in ivy bloom, with the highest percentage of flowers open (32.3%) also on the week of 30 September. There was a significant relationship between female activity and percentage of flower bloom (GLMM, $F_{1,61}$ =37.6, P<0.001). Female activity also significantly changed with week (GLMM, $F_{1,59}$=39.8, P<0.001) following a quadratic pattern. Male activity peaked when on average only 20% of flowers were in bloom (Fig 4).

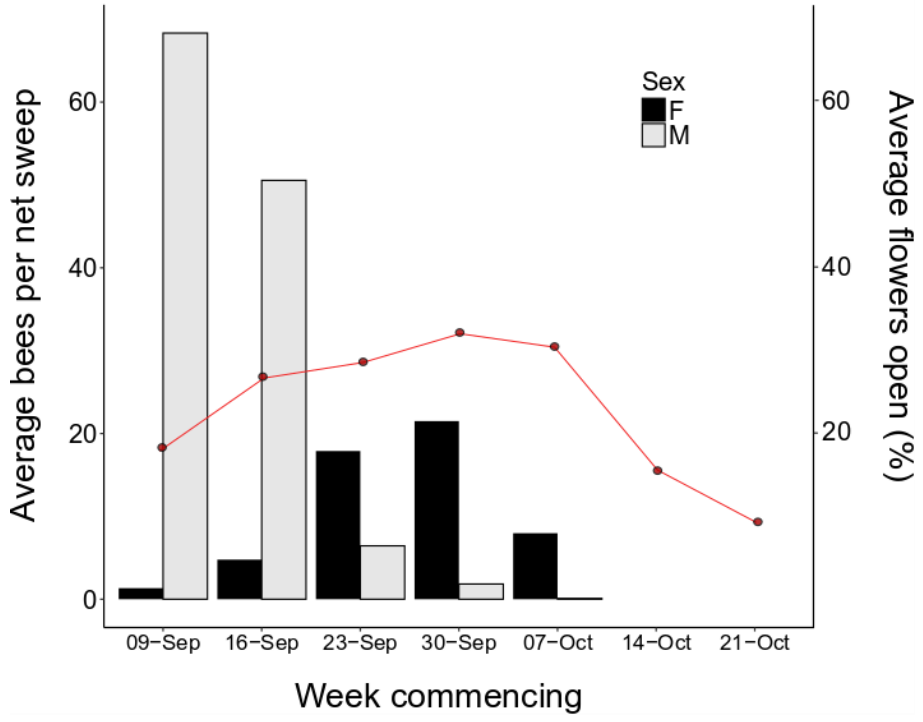


Fig 4: Mean number of *C. hederae* caught per sweep with an insect net per week. The red line indicates the average number of ivy flowers open for the corresponding week. Data has been pooled from Lewes and Falmer for both bee activity and flower bloom. No *C. hederae* were caught on the weeks of 14 and 21 October

4.4.4 Insects on ivy flowers

A total of 14 insect groups and 1565 insects were recorded foraging on ivy flowers during the survey period (weeks beginning 2 September to 21 October) across the two survey sites (SU Campus and Falmer). The most common species was *C. hederae* which accounted for 25.8% (403/1565) of sightings, followed by hover fly species (23.5%, 367/1565) and *A. mellifera* (22.9%, 359/1565). Few other bees were seen. Bumblebees (Hymenoptera: Apidae) comprised from 0-3%, with an average of 1% of insects seen per week (Table 2). Only one other bee, genus *Andrena* (Hymenoptera: Andrenideae), was seen throughout the whole survey period.

At the beginning of the survey period the *C. hederae* seen on ivy flowers were mainly male (71% week of 2 September and 80% week of 9 September). Subsequently, the proportion of males reduced with females being the most abundant. In the week of 16 September, females accounted for 66% of *C. hederae* seen, then averaged 95% in the remaining survey weeks. Initially most females seen did not have visible pollen loads in their scopa (first two survey weeks average of 16.7% across sites, Fig 5a). This increased throughout the survey period with an average of 88.0% of females with pollen between 16 September and 21 October, reaching a peak of 100% by the final week (21 October).

Colletes hederae sightings reached their peak on the week of 30 September with over 50% of insects being *C. hederae* across both sites (Fig 5b). This also corresponds with the peak ivy bloom and when females were most active at the aggregations (Fig 4). After this week, the proportion of *C. hederae* decreased to 5% by the week of 14 October and 1.5% on the final survey week (21 October). Overall, Falmer had a higher proportion of *C. hederae* compared to the campus site (30% vs 21%).

By contrast, although *A. mellifera* proportions did fluctuate between 10% and 33%, averaging 23% per week, they did not show the clear increasing and then decreasing trend seen by *C. hederae*. The proportion of *A. mellifera* workers collecting pollen was higher later in the survey period, with an average of 84% for the last two survey weeks (14 -21 October). Prior to this 51% of females were seen collecting pollen (Fig 5a).

Table 2: The insect groups identified foraging on ivy during surveys at A) Falmer B) Campus. The average weekly proportion for each insect group or species is shown along with the total proportion for the whole survey period. Data was collected from a total of 8 samples days and *N* indicates the total number of individuals recorded over the whole survey period.

A) Falmer		
Species/Group	Average weekly Proportion (% ± SE)	Total Proportion (%)
Colletes hederae	29.8 ± 7.94	30.1 *N=256*
Apis mellifera	33.4 ± 4.20	31.4 *N=267*
Bombus	1.13 ± 0.35	1.06 *N=9*
Vespula vulgaris	11.6 ± 3.92	10.6 *N=90*
Syrphideae (Hoverflies)	16.44 ± 4.58	18.6 *N=158*
Diptera (Other flies)	7.94 ± 2.38	7.18 *N=61*
Lepitoptera (Butterflies)	1.19 ± 0.53	1.06 *N=9*
B) SU Campus		
Colletes hederae	19.3 ± 5.05	20.6 *N=147*
Apis mellifera	14.6 ± 2.95	12.9 *N=92*
Bombus	0.25 ± 0.25	0.28 *N=2*
Apoidea (other bees)	0.001 ± 0.001	0.14 *N=1*
Vespula vulgaris	25.0 ± 6.07	23.5 *N=168*
Syrphideae (Hoverfly species)	27.8 ± 6.48	29.3 *N=209*
Diptera (Other flies)	12.9 ± 1.09	12.3 *N=88*
Lepidoptera (Butterflies)	1.13 ± 0.99	1.26 *N=9*

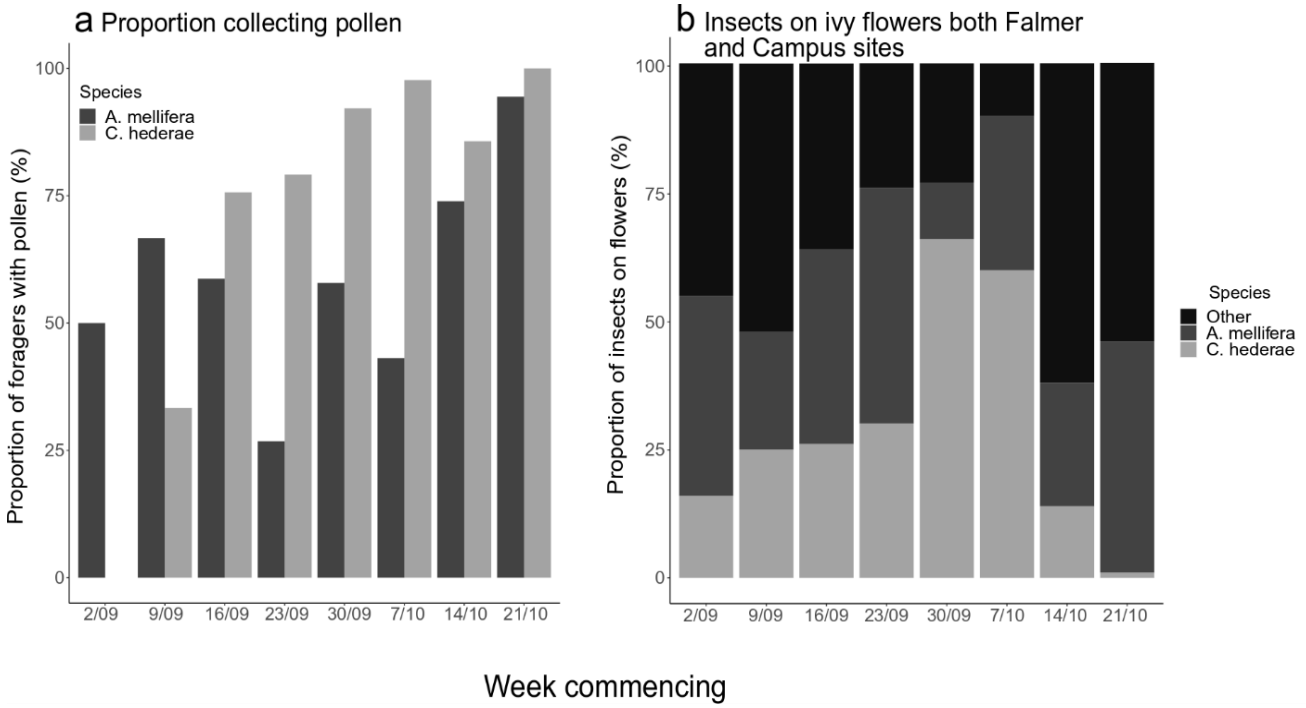


Fig 5: a) Proportion of female *A. mellifera* and *C. hederae* which were seen to be carrying ivy pollen in their scopa, b) proportion of different insect taxa seen foraging on ivy flowers with both SU Campus site and Falmer site combined. At the SU Campus site weeks 23 September, 14 and 21 October fewer than 100 insects were surveyed. All other weeks 100 insects were counted at each site.

4.5 Discussion

Our results show the importance of ivy, to the ivy bee, *Colletes hederae*. Almost all the pollen grains identified from females returning to the nest aggregation were ivy (98.5%). In addition, the phenology of female nesting and foraging activity took place exclusively within the ivy bloom period with peak activity at the aggregation and on flowers both coinciding with peak bloom. In comparison, although honey bees, *Apis mellifera,* collected a large proportion of pollen (90%) from ivy during the same period, this was significantly lower than for the *C. hederae*. In addition, *A. mellifera* pollen collection on ivy did not show such a tight relationship with the ivy bloom as that of the *C. hederae*.

4.5.1 Colletes hederae pollen composition

The foraging behaviour of *C. hederae* in this study can be considered eclectic oligolecty which is defined as 95% or more of the grains collected belong to the same genus and found in 95% or more of the pollen loads (Müller & Kuhlmann 2008). Previous work studying

pollen from *C. hederae* females has shown they have a high association with ivy but have also highlighted how the proportion of ivy pollen collected can vary, depending on the floral resources available. Bischoff et al (2005) sampled 15 pollen loads and 20 cells of *C. hederae* from aggregations in Dirmstein, Germany; all samples were 100% ivy pollen. In contrast, Müller & Kuhlmann (2008) examined pollen taken from museum specimens and found that only 78% of samples contained pollen from only ivy, and that on average it was 88% of the sample. However, inferring diet breadth from museum specimens can, for example with *Andrena dunninigi*, result in higher foraging diversity than is seen in observations or from pollen collected from living females (Johnson 1984). The most common other species identified in *C. hederae* pollen samples included *C. vitalba* and *R. fruticosus* and for the aggregations at Lewes and SU Campus the proportions of these species were significantly higher at the beginning of the females flight season. This flexible foraging behaviour is seen in many oligolectic species (Bischoff 2003, Ritchie et al 2016, Ogilvie & Forrest 2017) and has been previously reported for *C. hederae* and the closely related *C. succinctus* (BWARS 2011). Westrich (2008) identified pollen from multiple species during the first half of *C. hederae* flight period. However, once ivy began to bloom *C. hederae* began to collect pollen solely from ivy (Westrich 2008). In the week where *C. hederae* samples from the Lewes aggregation contained the smallest proportion of ivy (92%), ivy flowers had still not reached their peak bloom, with on average patches only having 20% of flowers open. This indicates that *C. hederae* females will collect pollen from other species.

There was a difference in the proportion of ivy present in samples among the aggregations, with bees nesting in Lewes having significantly less (97% vs 99%). The Lewes aggregation was in a suburban setting, and although there was a high abundance of ivy (61 two-meter patches surveyed) the densest patches were located approximately 150m from the aggregation. This is further than for both Falmer (<50m) and SU Campus (100m). This may have resulted in individuals reaching other flower species before locating an abundant source of ivy.

4.5.2 Phenology

Although *C. hederae* females did gather pollen from other species, our results indicate that female activity is highly correlated with and is contained within the ivy bloom period, with female activity peaking at the same time as the ivy bloom. By contrast, male activity did not synchronise with ivy bloom, peaking when only 20% of flowers were open on average. This

is probably due to males not having to collect pollen for larval food, therefore there is less reliance on the specific host plant. Males in this study were seen foraging on a range of flowers in August before the ivy bloom, including the wild flowers, bristly oxtongue (*Helminthotheca echioides*) and great willowherb (*Epilobium hirsutum*) and garden flowers, *Helenium* variety Sahin's Early Flowerer, indicating less floral specialisation. Differences in male and female foraging preferences are seen in other oligolectic species (Ritchie et al 2016) and may also be due to this reduced synchronisation between males and the main host plant species.

4.5.3 Comparison of pollen composition between C. hederae and A. mellifera

Colletes hederae pollen samples contained significantly higher proportions of ivy compared to *A. mellifera*, (98% vs 90%). Previous work on ivy has shown its importance to *A. mellifera* colonies in autumn. Garbuzov and Ratnieks (2014) found the similar result of ivy being 89% of pollen pellets in the months of September and October. Even when *A. mellifera* are most reliant on ivy, when there is not much else to forage on near the end of autumn, *C. hederae* still collects proportionally more ivy pollen (Fig 3a).

Individual *A. mellifera* workers are known to be flower constant (Grant 1950), usually foraging on only one species of flower per foraging trip. This was seen in our results with all pellets containing only one species of pollen. This is in contrast with *C. hederae* where only 65% of samples contained a single species. It has been suggested that whether a bee remains constant or not on a foraging trip depends on time and distance between it leaving a flower and then encountering another of the same type (Chittka et al 1997). At the beginning and end of the ivy bloom period, patches would often have inflorescences where only a few flowers were open, with these spread throughout the patch. The two main other sources of pollen for *C. hederae, C. vitalba* and *R. fruticosus*, often grow along walls and in hedges and in wooded areas and were often found nearby or intermingled with ivy, which would facilitate multi-species foraging. Often, only a small number of pollen grains from these other plant species were found in samples, perhaps indicating collection from only a few flowers. It is possible that pollen collection was incidental when the female was primarily collecting nectar. However, ivy flowers produce large amounts of nectar, which suggests that other flowers do not need to be visited to gather it. However, other species of oligolectic bees have been seen to have a wider breadth of diet for nectar other than their host pollen plant (Wcislo

and Cane 1996, Cane and Sipes 2006). Surveys of these other plant species would be required to identify if females are seen foraging for nectar.

4.5.4 Insects foraging on ivy flowers

Colletes hederae was the most numerous insect foraging on ivy flowers across the survey period (26%). However, this proportion varied between weeks, depending on the ivy bloom. The proportion of *A. mellifera* each week also fluctuated. However, in contrast to *C. hederae*, *A. mellifera* proportions did not show any consistent trend with the ivy bloom. With *C. hederae*, the proportion of females collecting pollen increased as more ivy flowers came into bloom. This was also the case with *A. mellifera*, with the proportion collecting pollen increasing in the final 3 weeks of the survey period. This increase is most likely due to the lack of other available flowers, rather than a specific reliance on ivy, and the need for pollen to rear brood and to store. Both *A. mellifera* and *C. hederae* collect pollen to feed brood. However, *C. hederae* females must first mate and excavate a nest before they have larvae to provision (Schäffler & Dötterl 2011), unlike *A. mellifera*. Before this point pollen collection is not required, but energy from nectar is needed for nest digging and cell building, perhaps explaining the low proportions of females in ivy flowers collecting pollen early in the survey period.

There was a difference in the proportion of *C. hederae* seen foraging on ivy between the sites with Falmer having a higher proportion compared to SU Campus (30% vs 21%). The Falmer aggregation was the largest of the three in this study. In 2018 it was estimated to have over 4000 nests, versus only 115 at SU Campus (Hennessy et al 2020). This suggests proximity to large nesting sites is an important factor in determining the presence of foraging *C. hederae*. This makes sense as *C. hederae* have an estimated foraging distance of approximately 500m (Greenleaf et al 2007), meaning local densities of both ivy and *C. hederae* will have a major effect on the numbers foraging on a specific patch.

4.5.5 Potential for competition

Colletes hederae has experienced a large geographic range expansion in the last 20 years throughout Western Europe (Dellicour et al 2014). This expansion includes Britain, with the first sighting of *C. hederae* in Dorset in 2001 (Cross 2002).

Colletes hederae is an invasive species to the UK, and as seen with the Falmer aggregation in this study, can nest in high densities and be the most abundant insect on ivy flowers. Previous

surveys of insects on ivy flowers, completed in 2011 in the same location found *C. hederae* to be only 3% of the total insects recorded (Garbuzov & Ratnieks 2014). The proportion seen in this study (26%) is much higher than in 2014, perhaps indicating how quickly this species can build up to become extremely abundant, approximately equalling *A. mellifera* in an area with numerous hives. This raises the question as to whether *C. hederae* may be harmful to native species via competition for floral resources. In this study we identified 14 different insect groups. Of these, only bees collect pollen to provision nests. Hoverflies (Diptera: Syrphidae) consume small amounts of pollen for their own use as an adult (Hickman et al 1995) but *Vespula vulgaris* (Hymenoptera: Vespidae), butterflies (Lepidoptera) and other flies (Diptera) consume only nectar (Norris 1936, Larson et al 2001). These taxa will utilise much less of the ivy resources compared to *C. hederae* and *A. mellifera.* However, competition may still occur for nectar resources, as *C. hederae* will not only collect pollen for developing larvae, but also nectar. There was no evidence of antagonistic behaviour between foraging *C. hederae* and other flower visitors to ivy. However, exploitative competition through reduction of resources is seen in other systems (Balfour et al 2015, Wignall et al 2020). Evidence of an invasive solitary bee influencing a local pollinator species has been seen with introduced *Anthidium manicatum* (wool carder bee), where native *Bombus impatiens* do not forage on patches where *A. manicatum* are present (Graham et al 2019). However, *A. manicatum* males are highly aggressive, likely actively excluding other foraging insects, which is not seen with *C. hederae*. Although floral resources are relatively limited when *C. hederae* are active in the autumn (Couvillon et al 2014), ivy is highly abundant, and most other bee species are no longer active (Goulson 2003), perhaps reducing competition. The number of foraging *C. hederae* on ivy were similar to the eusocial *A. mellifera,* a species which some research suggests is capable of outcompeting wild bees through exploitative competition due to their large numbers (Henry and Rodet 2018) but which can itself be outcompeted in this way (Balfour et al 2015). Considering the strong dispersal ability of the species, the fact its parasites are not yet found in the UK (Dellicour et al 2014) and that females can produce 3x as many offspring as the closely related *C. halophilus* (Danforth et al 2019), an increase in abundance and range of the species is likely to continue. This has already been seen in Europe, with one study showing that the number of grid cells occupied by the species in Europe had increased by 6 to 7 fold between 2001 and 2010 (Dellicour et al 2014). This rapid expansion is perhaps surprising, due to the species apparent reliance on a single plant species. However, its dependence is on a common plant species and the warmer summers much of Europe has experienced over the last few decades may have contributed to the production of

more abundant and longer lasting flower stands of ivy as it reproduces vegetatively in colder climates (Iversen 1944). More research is required to understand how the increasing number of this invasive species may impact the local pollinator community. This is especially true due to the expanding nature of the species throughout Britain, and other parts of its range.

4.5.6 Conclusion

In conclusion, ivy pollen has been identified as a crucial floral resource for *C. hederae* in this study. This is shown through the high proportion of ivy pollen collected by females, the matched phenology of female activity and ivy bloom intensity, and that *C. hederae* are the most common insect foraging on ivy during their flight season. This study has only focussed on three locations all in the South of England, well within the species expanded range. Future work in a range of locations is needed to identify if *C. hederae* foraging patterns are consistent throughout where it is found. Also, future research on the potential of competition between this invasive and our native species is required to determine what impact, if any, their presence is having.

Chapter Five: Gone with the wind: Effects of wind on honey bee visit rate and foraging behaviour

5.1 Abstract

Wind is an important yet understudied environmental influence on foraging behaviour. We investigated the direct and indirect effects of wind on foraging worker honey bees, *Apis mellifera.* Bees were trained to an array of artificial flowers providing nectar rewards in a location sheltered from natural wind. To examine the direct effect, fans produced four different wind speeds between 0 and 3 m/s at three different flower spacings: 5 cm (flowers touching) and 10 cm and 20 cm (flowers not touching). To examine the indirect effect of wind moving flowers, flowers were moved 10 cm at three frequencies between 50 and 110 cycles per minute at zero wind speed. Foraging behaviours were examined including: number of successful flower visits, time flying, search time on a flower and hesitancy to take off. Bees visited significantly fewer flowers with increasing wind speed which was caused by a significant increase in hesitancy to take-off. This difference in flower visits among wind speeds was highest at the 20 cm spacings. Flower movement had no effect on foraging rate however there was a significant positive relationship between flower movement and the total time flying. This was counterbalanced by a significant reduction in time spent searching for the nectary after landing on a flower at the higher flower frequencies. Our results suggest that it is the direct effect of wind on hesitancy to take off which has the greatest effect on honey bee foraging rate.

5.2 Introduction

Animals show many adaptations that enhance foraging effectiveness and efficiency (Stephens and Krebs 1986, Boyd et al 1997, De Knegt et al 2007). Foraging is also affected by many environmental factors, which can exert their influence both directly and indirectly. Direct examples include the effects of light and temperature on the time of day at which animals forage (Fraser et al 1995, Cannell & Cullen 1998, Aublet et al 2009), and can help, hinder or even prevent foraging altogether (Radford et al 2001). Indirect examples include light causing increased predation risk, with white footed mice ceasing to forage much sooner on clear, lighter evenings (Orrock and Danielson 2009). Temperature can cause fluctuations in prey availability, forcing bats to travel further to warmer feeding sites (Arbuthnott & Brigham 2007).

Wind is an important environmental factor that can both help and hinder foraging. The wandering albatross benefits from favourable tail and side winds whilst traversing the ocean (Weimerskirch et al 2000 & Weimerskirch et al 2002). By contrast, leaf cutter ants walk 55% more slowly and are blown off their path more in windy conditions (Alma et al 2016). Wind influences flight during migration and travel between foraging sites in birds (Liechti 2006 & Wakefield 2009). It can also directly affect foraging decisions and success. Sandwich terns decrease their foraging rate with increasing wind speeds (Taylor 1983) and glaucous gulls vary their foraging strategy with wind speed to maximise energy gain and minimise risk of injury (Gilchrist et al 1998). Wind can also influence foraging indirectly by altering the presence of prey. Insect abundance increases near strips of vegetation and woodland as they provide a buffer from wind (Whitaker et al 2000) and following summers of high wind speeds barn swallows had lower breeding success due to fewer insects (Møller 2013).

Wind is expected to be especially challenging for flying insects due to their small body size. Flight is energetically expensive (Reinhold 1999), and this cost increases with wind speed in birds (Tucker and Schmidt- Koenig 1971). With increasing wind speeds energy expenditure increased by 30% in euglossine bees due to leg extension to help with stabilisation (Combes et al 2009). High wind speeds make landing more difficult and less controlled in bumblebees, resulting in higher impact landings (Chang et al 2016). Wind may provide additional challenges to flower visiting insects, especially bees, who make many small flights between individual flowers or inflorescences, with estimates for honey bees of between 250 and 1446 flower visits per trip (Ribbands 1949, Goodwin et al 2011). Even a small increase in the time taken to travel

between and land on flowers caused by wind-induced delays would be multiplied hundreds or even one thousand times (Couvillon et al 2015) per hour of foraging.

One indication of the effects of wind on bee foraging is a reduction in the number of foraging honey bees present at foraging sites with increasing wind (Pinzauti 1986, Comba 1999, Vicens and Bosch 2000). Surprisingly, however, there appear to be no data directly measuring the effect of wind speed on the foraging rates of individual bees or how wind may directly or indirectly affect foraging, for example by causing flowers to move and to be harder to land on.

The aim of this study was to determine the effect of wind speed and flower movement on the foraging behaviour and rate of flower visits of honey bee workers, *Apis mellifera.* We used an array of artificial flowers and wind generated by fans to decouple the direct effect of wind on the bee versus indirect effects caused by wind-induced flower movement. In Experiment 1 we used a 2-way design of 4 wind speeds at three flower spacings to investigate the direct effect of wind on foraging rate. In Experiment 2 we investigated the indirect effect by moving the artificial flowers at different rates to simulate flower movement caused by wind.

5.3 Materials and methods

5.3.1 Study site and organism

We studied free flying honey bees (*Apis mellifera*) foraging on artificial flowers in arrays located in the porch of the workshop building beside the main apiary at the Laboratory of Apicultural & Social Insects, on the campus of the University of Sussex. The outside walls of the porch were covered in clear polycarbonate plastic which resulted in the interior being well illuminated but windless. Data were collected in October and November 2017 for experiment 1 and August 2018 for experiment 2 on days sufficiently warm for flight (10-24°C). All bees studied had flown from their hives to feed on artificial flowers of the same design as those being used in the experimental arrays, located 1-2m from the porch entrance. This indicated their readiness to forage under the prevailing conditions and allowed them to learn how to access the sugar solution in the centre of each flower.

5.3.2 Artificial flower design & construction

The artificial flowers were based on those used by Grüter et al (2011) and did not move in the wind. This allowed us to study the direct effects of wind on bee foraging without any flower movement (Experiment 1) or, by manually moving the flowers, to separately assess the indirect

effect of flower movement without wind (Experiment 2). The petal area was an eight pointed 4x4 cm radially symmetrical yellow star with a black cross consisting of two 1 mm black lines crossing in the centre and acting as a nectar guide (Fig 1). The flowers were laser printed on white paper and laminated with plastic. To create a nectary to hold the sucrose solution, the final 5 mm of an Anachem 200 µl plastic pipette tip was fitted into a hole cut where the nectar guides crossed until the upper edge was flush with the petal surface. The lower hole was plugged with Blue Tack © to prevent syrup placed into the nectary from draining away. The 5 mm nectary depth was chosen as this is well within the 6.6 mm reach of *A. mellifera* (Balfour et al 2013). The petal part of the flower was elevated on a 10 cm stalk made from half a plastic drinking straw.

5.3.3 Arrangement of flower arrays

Artificial flowers containing 3 µl of 45-50% sucrose solution were set up on a 65 x 75 cm plywood base with three different spacings between neighbouring flowers, measured from the centres: 5 cm (flowers touching), 10 cm, and 20 cm apart. The number of flowers per arrangement was 42 (5 cm), 20 (10 cm) and 8 (20 cm). We tested different flower spacings as bees are known to forage in the most efficient way to reduce foraging costs (Pyke 1979 & Pyke 1978) with flying being more energetically costly than walking (Heinrich 1975). With one spacing giving the option of walking between flowers we were also able to test the hypothesis that bees are more likely to walk in higher wind speeds. Having different flower spacings also mimics a more natural system, with plant species having a diverse range of distances between neighbouring flowers (Balfour et al. in prep).

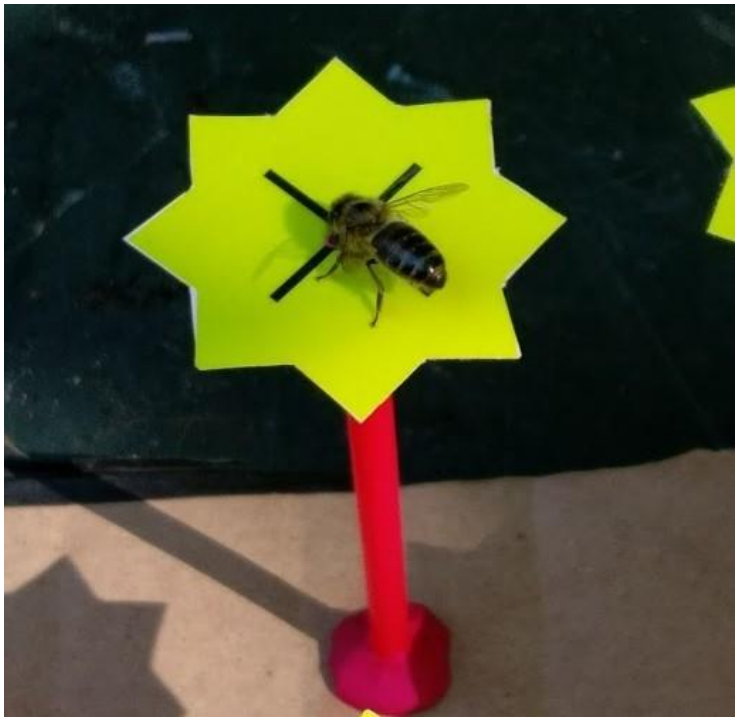


Fig 1: Honey bee foraging on an artificial flower as used in this project. In the middle of the black cross (acting as a nectar guide) the end of a pipette tip is inserted flush with the face of the flower and contains 3µl of 50% sucrose solution.

5.3.4 Training bees to flowers

Four hives from the adjoining apiary were trained to the artificial flower patch using a glass nectar feeder, a small jar filled with nectar solution, placed on a grooved base where worker bees could take syrup (Seeley 1989). This was placed outside the porch, next to filled artificial training flowers, and removed when enough bees were attracted to the feeder. When bees had located and were foraging successfully from the artificial training flowers located outside the porch, they were given access to the experimental set up inside for data collection. This was to ensure bees being used in the experiment knew how to access the nectar from the artificial flowers so that only foraging behaviour, and not learning, was tested.

5.3.5 Wind generation

In experiment 1 each foraging bee was exposed to one of four wind speeds as it foraged on one of the arrays. As data collection took place in the sheltered porch there was no external wind. Three fans (Olypa H-28401 Electrical 16-Inch Oscillating Pedestal Stand fan) were placed

opposite the flower array at a height of 1 m, facing the long side of the array to minimise wind variation within the patch. The wind speeds tested were (0) No wind, (1) Wind speed 1.6-1.9 m/s, (2) Wind speed 1.9-2.4 m/s (3) Wind speed 2.6-3.0 m/s. Wind speed was adjusted by moving fans closer or further away to the patch (1.3 m for wind speed 1, 0.9 m for 2, 0.6 m for 3) and increasing the speed setting on the fan. Wind speeds were measured using a hand-held anemometer (HOLDPEAK 866B Digital Anemometer). During the no wind condition a fan was placed facing away from the flowers and turned to setting 1 to act as a control for any non-wind effects of the fans.

5.3.6 Experiment 2- Indirect effect of wind on bees

Experiment 2 investigated the indirect effects of wind through flower movement on bee foraging using the same artificial flowers and location as in experiment 1. There was no wind and only the 10 cm flower spacing was studied. To cause flower movement the table holding the flower array was placed on wheels and manually moved backwards and forwards at different frequencies; 0, 50, 80 or 110 cycles per minute, with each cycle being forwards and backwards to the initial position. The distance at which the flowers moved was fixed at 5 cm either side of the table, which resulted in a total of 10 cm travelled per cycle. The speed of movement was controlled using a metronome and the distance was controlled using wooden blocks fixed to the floor. Two people would move the table in a smooth and controlled manner. 110 cycles per minute was chosen as the maximum movement rate as above this bees often abandoned the foraging attempt and it became harder to control the movement of the table. The lowest movement of 50 cycles per minute was chosen as previous work has found this rate of flower movement challenging for flying insects (Alcorn et al 2012).

5.3.7 Foraging trials on individual bees

The same method was used in both experiments. A test bee that had been trained on the artificial flowers entered the experimental area through the door of the porch which was then closed. Once the bee landed on its first flower it foraged without interruption for 90 seconds. The bee was then caught, weighed, and marked with a paint dot so that previously tested bees could be excluded from subsequent trials. The flowers it had foraged on were then removed and replaced with refilled flowers to ensure equal volumes of nectar for each trial and so that no odour marking was present on the flowers. Flowers were not replenished during the trial to avoid any disturbance to the foraging bee and to mimic a natural ecological system where previously visited flowers would have little to no nectar present (Stout & Goulson 2002). Corollas were

later washed in water, dried and then reused on subsequent days. The order of the trials was chosen randomly to give a minimum of 10 complete trials for each wind speed and movement rate combination. If a bee did not forage without interruption for the whole 90 seconds it was not included in the analysis.

5.3.8 Video recordings and decoding

Each trial was recorded using a Sony HD handheld camera (model HDR-CX115, 24 fps) on a tripod 1.5 m from the array ensuring all flowers were in shot. By playing back the videos frame by frame on a computer the following information was obtained: number of flowers successfully visited (the bee landed on an unvisited flower and was seen to take nectar), total time spent flying, time flying per flower (total flight time divided by the number of flower visits), probing time per flower (time when the bee was actively seen to be taking syrup, identified through the pulsing of the abdomen, divided by number of flowers successfully visited), search time per flower (the time a bee spent on a flower before she started probing for nectar, divided by flower visits) and hesitancy to take off per flower (the time a bee spent on a flower once she had finished probing for nectar before taking off divided by successful flower visits). The 5 cm spacing (where flowers were touching) was excluded from the search time and hesitancy analysis because bees usually walked between flowers making identifying these behaviours unquantifiable in a way consistent with other spacings in which bees always flew from flower to flower

5.3.10 Data analysis and statistical methods

Statistical tests were performed in R 3.3.3 (R Development Core Team, 2009). For all variables both linear, quadratic and general linear models with differing family link functions were tested for best fit. Models were compared using AIC values and tested against model assumptions. Where possible dependent variables were transformed for normality. For experiment 1 search time per flower, probing per flower and hesitancy to take off per flower were log transformed and successful flowers and flight timer per flower were square root transformed. For experiment 2 all variables except probing per flower were log transformed. Transformations were decided by examining the distribution of the data and checking for normality once transformed.

Linear regression models were used to test the following behaviours as dependent variables for experiment 1: the square root of number of flowers visited to take nectar, the log of search time per flower, log of probing per flower and the square root of flight time per flower. A general

linear model with a quasipoisson distribution and log function was used to test the total flight duration due to the non-normal distribution of the data. A quadratic model was used to test the log hesitancy to take off from a flower. Multiple comparisons between the different flower arrangements were made using the general linear hypothesis method with the Tukey contrast (Hothorn et al 2008) where appropriate.

For experiment 2 linear regression models were used to test the number of successful flowers visited to take nectar, time probing per flower and the log of the remaining behaviours (search time per flower, hesitancy to take off, flight time per flower and total flight time).

Normality was tested using the Shapiro Wilk test. Wind speed and flower arrangement were the explanatory variables for Experiment 1 and flower movement frequency for Experiment 2. The interaction between the explanatory variables was included in the all models for Experiment 1 and the average of each wind speed range was used, these were: 0, 1.75, 2.15 and 2.80m/s.

5.4 Results

5.4.1 Experiment 1

A total of 124 bees were studied, 10 or 11 in each of the 12 combinations (four wind speeds x three flower distances).

5.4.1.1 Number of flowers successfully visited

The number of flowers successfully visited in 90 s ranged from one to 13 and was negatively correlated with wind speed (linear regression, $F_{1,120}$=13.98, P<0.001, Fig 2A, R^2=0.55) with a 37% reduction from Wind 0 to 3 (2.8m/s) when averaged over the three flower spacing's (Wind 0; $\bar{X}$=5.45 ±0.45, Wind 3; $\bar{X}$=3.73 ±0.50). This reduction was proportionately greater, 77%, for the greatest flower distance, 20 cm, versus 17% when flowers were touching. Bees visited fewer flowers when they were spaced further apart (linear regression, $F_{2,118}$=54.8, P<0.001, R^2=0.43). The interaction between flower spacing and wind speed was not significant (linear regression, $F_{2,120}$=0.455, P=0.642). Tukey *post hoc* tests show that the number of flowers visited significantly differed between all three spacing's (Table 1).

5.4.2.2 Search time per flower

The time taken to start imbibing nectar following landing averaged 3.14 seconds per flower and increased with wind speed (Fig 2B), but not significantly (linear regression $F_{1,72}$=3.79, P=0.054). Neither flower spacing (linear regression $F_{1,71}$=3.18 P=0.079) nor the interaction between spacing and wind speed (linear regression $F_{1,70}$=1.30, P=0.258) had a significant effect on search time (Table 1).

5.4.1.2 Probing per flower

There was no effect of wind speed or flower spacing (Fig 2C, linear regression $F_{1,122}$=0.896, P=0.442, $F_{2,120}$=1.26, P=0.288) or their interaction ($F_{2,118}$=0.530, P=0.590) on the time a bee spent probing for nectar.

5.4.1.3 Hesitancy to take off per flower

The hesitancy to take off from a flower ranged from 0.05 to 54 seconds and when examined per flower was positively correlated with wind speed (quadratic regression, $F_{1,74}$=11.32 P<0.001, Fig 2D). Between W0 and W3 (2.8m/s) there was a 97% increase in hesitancy to take-off combined across all spacings (Wind 0; $\bar{X}$=7.59(s) ±1.18, Wind 3; $\bar{X}$= 22.02(s) ±3.12) and when examined per flower this increased to 139% (Wind 0: $\bar{X}$=2.49(s) ± 0.15, Wind 3: 13.93(s) ± 2.94). Flower spacing and its interaction with wind speed was not significant (quadratic regression $F_{1,73}$ =0.236 P=0.628; $F_{1,73}$=0.115, P=0.914) (Fig 2D).

5.4.1.4 Flight time per flower

Wind speed did not significantly influence flight time per flower (linear regression $F_{1,122}$=0.267, P=0.607). The interaction between wind speed and flower spacing was not significant (linear regression $F_{2,120}$=2.748, P=0.068), flower spacing alone was (linear regression $F_{2,118}$=61.79, P<0.001, R^2=0.49). Tukey post hoc tests showed that flight time per flower significantly differed between all spacings (Table 2). The spacing where bees spent the least amount of time flying per flower was 5 cm, where on average across all wind speeds bees only flew for 0.49 s. This is less than half the amount of time bees flew per flower when compared to the 20 cm spacing (Fig 2E). When flowers were touching bees would often walk between them instead of fly at all wind speeds, and when only testing the 5 cm spacing there was no relationship between wind speed and flight time per flower (general linear model, $F_{1,40}$=2.64, P=0.112).

5.4.1.5 Total flight duration

There was a significant negative relationship between wind speed and total flight duration Fig 2F, (general linear model, $F_{1,122}$=5.71, P=0.018). Flower spacing alone also affected the total flight duration (general linear model, $F_{2,120}$=64.78, P <0.0001) with Tukey *post hoc* tests showing this differed between 5 cm spacing and the other two spacings, although not between 10 cm and 20 cm (Table 2). Total flight duration was five times less for the 5 cm spacing than 20 cm (means of 4.6vs 23.7 s). The interaction between wind speed and flower spacing was not significant (general linear model, $F_{2,118}$=1.07 P=0.347).

Table 1: Tukey post hoc test results for flower spacing comparisons for number of flowers successfully visited, flight time per flower and total flight time.

Behaviour	*Comparison (cm)*	*Estimate*	*Std. Error*	*Z value*	*P value*
Number of flowers successfully visited	10--5	-0.621	0.092	-6.729	<0.0001
	20--5	-0.950	0.092	-10.301	<0.0001
	20--10	-0.330	0.093	-3.551	0.001
Flight per flower (s)	10--5	1.290	0.162	7.948	<0.0001
	20--5	1.701	0.162	10.486	<0.0001
	20--10	0.412	0.163	2.502	0.0314
Total flight time (s)	10--5	1.394	0.237	5.891	<0.0001
	20--5	1.634	0.231	7.062	<0.0001
	20--10	0.240	0.143	1.681	0.205

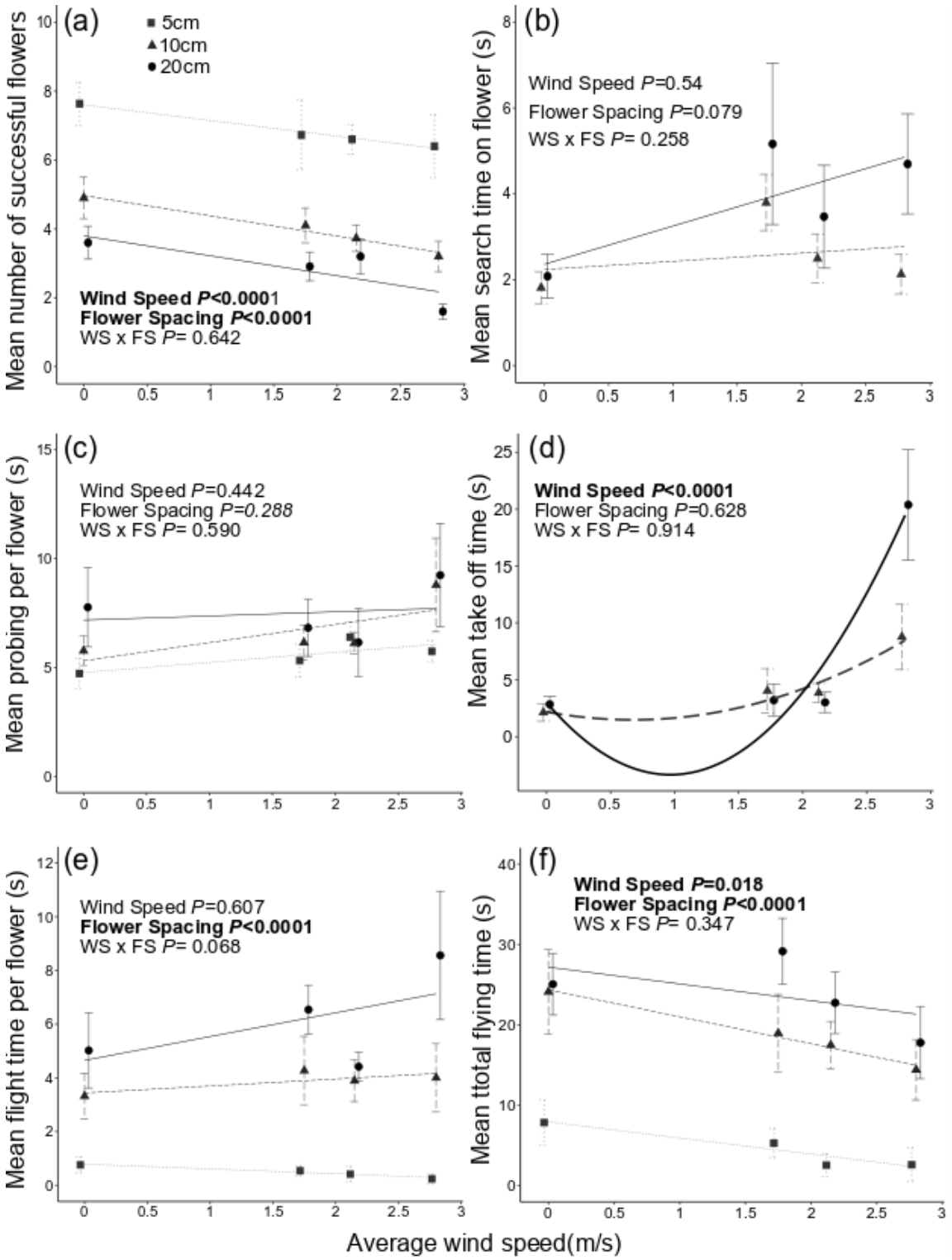


Fig 2: Effect of wind speed and flower spacing on honey bee foraging behaviour and performances on experimental artificial flowers. The lines show either linear or quadratic regression per flower spacing. Error bars show the standard errors and factors in bold are significant. For figures A,C,E and F *N*=124 for B *N*=74 and D *N*=77.

5.4.2 Experiment 2

A total of 41 bees were studied, 10 for each 0, 50 and 120 flower array cycles per minute and 11 for 80.

5.4.2.1 Number of flowers successfully foraged

Flower movement had no effect on the number of flowers successfully visited during the 90 second trials (Fig 3A, linear regression, $F_{1,39}$=1.70, P=0.20). The lowest was when flowers were stationary (mean=6.10 ±0.69), and the highest was at 110 cycles per minute (mean=7.80 ±0.61).

5.4.2.2 Search time per flower

As flower movement increased, search time significantly decreased (linear regression, $F_{1,39}$=6.89, P=0.012, Fig 3B). There was a 99% decrease in search time between stationary and 110 cycles, the highest rate (3.75 ±1.02, 1.26 ±0.18) Between 50 BPM and 110 BPM there was a 60% reduction in search time (2.35 ±0.72, 1.26 ±0.18).

5.4.2.3 Probing per flower

There was no relationship between probing time per flower and flower movement (linear regression, $F_{1,39}$=0.005, P=0.947, Fig 3C).

5.4.2.4 Hesitancy to take off per flower

There was no relationship between hesitancy to take off from a flower and flower movement (linear regression $F_{1,39}$=0.186, P=0.669, Fig 3D).

5.4.2.5 Flight time per flower

The flight time per flower significantly increased with flower movement (linear regression $F_{1,39}$=4.654, P=0.037, Fig 3E). Flight per flower was highest at 50 BPM and lowest when flowers were stationary (3.41 ±1.49 vs 1.93 ±0.68 s).

5.4.2.6 Total flight duration

There was a significant positive relationship between total flight duration and flower movement rate (linear regression, $F_{1,39}$=9.947, P=0.003). There was a 65% increase in total flight duration between when flowers were stationary and when they were moving at 110 BPM (12.9 ±8.3 vs 25.4 ±2.67 s). There was a 54% increase in flight duration between stationary flowers and when they were moving at the lowest rate, 50 BPM (Fig 3F).

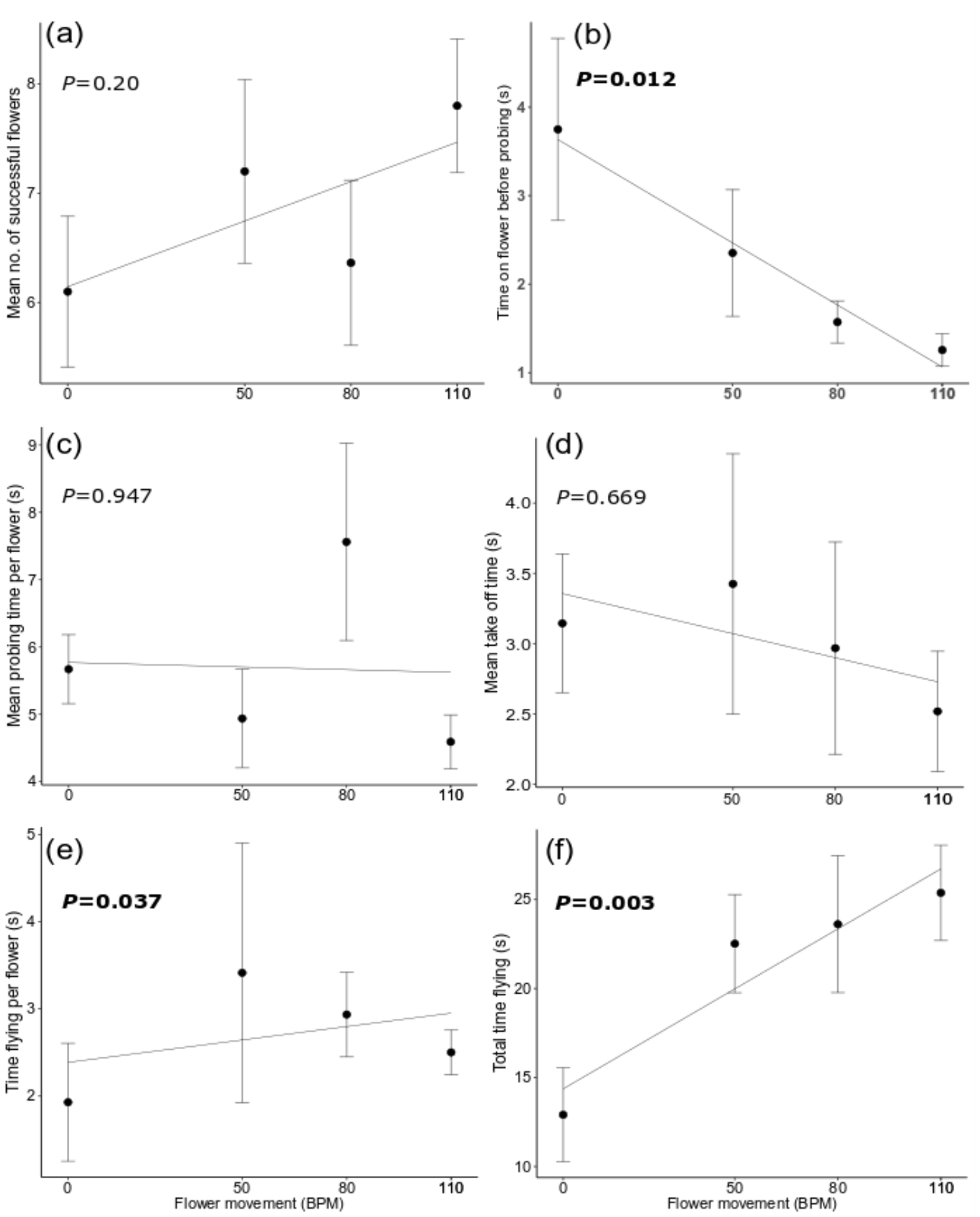


Fig 3: Effect of flower movement on honey bee foraging behaviour and performance on experimental artificial flowers. The lines show linear regression and standard error bars and values in bold are significant, $P<0.05$. For all graphs $N=41$.

5.5 Discussion

Our results show that wind speed, flower spacing and flower movement all influence honey bee foraging behaviour. By decoupling the effects of wind and flower movement our results suggest that, under the conditions studied, the direct effect of wind on foraging honey bees has more of an effect than the movement of the flowers themselves. This is shown by the significant reduction in forage rate with increasing wind speeds (Fig 2A) but not with an increasing rate of flower movement (Fig 3A). Both wind speed and flower movement were found to influence specific honey bee foraging behaviours. Unexpectedly a major effect of increasing wind was that honey bees hesitated for significantly longer when taking off from a flower when wind speeds were high. This was not the case for increased flower movement. Here, honey bees were found to spend significantly less time searching for the nectary once having landed on a flower with increased movement (Fig 3B) but did spend longer in flight (Fig 3E, Fig3F).

5.5.1 Direct effects of wind on honey bee foraging

The result that bees have a lower foraging rate with increasing wind speeds helps understand field based studies which show that in windy patches of flowers fewer bees are seen foraging (Pinzauti 1986, Vicens & Bosch 2000, Chapter Seven). Vicens and Bosch (2000) identified wind speed as a major environmental factor influencing the number of foraging *A. melifera* on apple trees and found that even when temperature and solar radiation levels were favourable, if wind speed was moderate foraging activity would stop. The reduction in the rate of flower visits by individual bees means that continuation of foraging might no longer be profitable at wind speeds higher than hose tested in this study. This has potentially negative repercussions for pollination services.

The unexpected and novel change in foraging behaviour that appears to have been mainly responsible for the reduction in flower visit rates was hesitancy to take off. After they finished imbibing nectar, bees took significantly longer to fly away from the flower at higher wind speeds (Fig 2D). There have been studies examining how insects alter flight orientation and movement (Ravi et al 2013, Crall et al 2016) with one even examining landing dynamics (Chang et al 2016). However, none have quantified difficulties in take-off and how this may influence overall foraging performance in honey bees. When initiating take off, flying animals

assume the correct body position to produce the most efficient wing angle required for flight (Jakobi et al 2018). This positioning will depend on the environmental conditions being experienced and as wind speed increases so does air turbulence (Ren et al 2018), making achieving the ideal orientation more difficult. Reduced ability to take off in higher wind speeds has been examined in other insect species (Haine 1955, Isaacs et al 1999) and may be due to this increase in air turbulence. In nature the wind speed varies and occurs in gusts. Possibly honey bees were waiting for a pause in the wind to take off, a behaviour observed in the parasitic wasp *Diachasmimorpha longicaudata* where individuals were seen to wait for a break in the wind before initiating flight and would increase flight activity in breaks between higher wind speeds (Messing et al 1997). Increased take off hesitancy may also be due to more rapid decline in body temperature with greater wind cooling. To initiate flight insects must attain a species -specific body temperature threshold which will then allow sufficiently rapid flight muscle contraction (Pasek 1988, Heinrich 1993). Even a small increase in wind speed can result in a significant lowering of body temperature, with the greatest cooling in wind speeds up to 3m/s (Church 1960 & Digby 1955). The increased hesitancy to take off seen in experiment 1 may have been due to bees needing longer to warm up the flight muscles required to take off. This increase in take-off time due to a decrease in body temperature has been seen in previous work examining sucrose concentration and body temperature in honey bees. When sucrose solution was less than 30% thoracic body temperatures would drop below that required for flight whilst bees were imbibing on the feeders, resulting in increased take off times of up to 3x compared to concentrations of 40% and above (Waddington 1990). The average ambient temperature throughout the study was 14°C, warm enough for flight however low enough to have an impact on honey bee thermoregulation (Esch 1988). If the increase in hesitancy seen in this project was due to thermal regulation, the same result might not have occurred in warmer ambient temperatures. Further experiments are needed to test these hypotheses to explain increased take off hesitancy with increased wind speed.

5.5.2 Indirect effects of wind on honey bee foraging through flower movement

Flower movement independent of wind speed had no impact on foraging performance, as bees visited flowers at a similar rate when flowers were moving or not. Field work on natural flowers found a similar result (Warren and James 2008). When stalks were manipulated to alter flower movement in the wind, flowers which exhibited moderate mobility had the highest visit rates, indicating flower movement is not necessarily a negative factor for flower visiting insects. Insects were also found to spend less time on flowers that moved more (Warren and James

2008). This mirrors our results. Honey bees were found to spend significantly less time searching for the nectary once having landed on a flower with increased rate of movement, resulting in less time spent per flower. Bee target detection is improved with the addition of motion and the use of colour cues (Kapustjansky et al 2010) perhaps resulting in improved landing accuracy, reducing the time required when on the flower to reach the nectary. Perhaps when flowers are moving it makes them easier to see and therefore easier for bees to land closer to the target (the nectary). If higher movement rates were examined this effect may change as bees might struggle more to land, missing the target.

Although honey bees spent less time on the surface of flowers with greater flower movement, the overall rate of flower visiting did not increase because flight times between flowers increased (Fig 3D). Bees may be taking longer to land when flowers are moving faster to maintain this landing accuracy. Foraging bumblebees utilise different strategies depending on the individual, some favouring accuracy over speed (Chittka et al 2003). When flower movement is increased bees may trade off costs and benefits of different parts of the flower handling sequence, such as longer flight time but shorter time spent searching for the nectary once on the flower.

The most important behavioural component influencing rate of flower visiting when wind was present was the increased hesitancy to take off. However, when flowers were moving this was not affected (Fig 3D). The absence of direct wind may make take off easier, or if bees' body temperatures decrease faster in higher winds they may not require the same time to take off in its absence.

Our study only examined honey bees foraging on artificial flowers. Future work could examine the same behavioural traits but on natural flowers and study other species to determine if the same effects are seen and to also identify if individual insects are better at adjusting to foraging in wind than others. Work on the artificial flowers could be continued by combining wind speed and flower movement to determine how the combination can influence honey bee foraging behaviour, as it would be rare for a bee to experience flower movement without wind. The wind speeds examined in this study are lower than the UK average (Statista 2019). However meteorological wind speeds are determined at a height of 10m in unsheltered locations (Met office 2019). A foraging honey bee would be amongst vegetation. Being close to the ground and sheltered would result in slower wind speeds (Crall et al 2017) more like the ones used in this study than those reported for the rest of the UK.

Bees provide a major ecosystem service through crop pollination, with honey bees accounting for approximately half of this service (Kleijn et al 2015). It is predicted that through climate change there will be a global increase in wind speed (Hosking et al 2018). This increase in windy weather could impact bees' pollination services in the future. Our study found an increase of just 2.75 m/s resulted in a 37% decrease in flower visits. With climate models predicting wind speed to increase by 1-5 m/s over the next 100 years in the UK (Robinson et al 2017) wind could impose a major reduction in pollination services (Tuell & Isaacs 2010), not just in honey bee dominated crops but potentially those pollinated by other species not examined in this investigation. This reduction in pollination efficiency is not only detrimental to pollination services but also for the bees themselves, as fewer flower visits per flight results in less food collected. Although the relationship between food influx into honey bee colonies and wind has not been directly examined, previous work has highlighted a reduction in foraging activity due to other poor weather conditions (rain and low temperatures) results in less food for the colony and a decline in brood production (Riessberger & Crailsheim 1997).

Bees and the threats they face (Potts et al 2010, Goulson et al 2015) are prevalent in the news today, especially in relation to climate change (Kharouba et al 2018,). Our study shows that an everyday weather phenomenon, the wind, can have a considerable effect on bee foraging. Although little can be done to control or stop the wind, efforts can be made to minimise its impact on pollinators. Placing hives in sheltered locations (Hunter 2015) and using natural wind breaks (Pinzauti 1986) are just some examples to help reduce the impact of wind on foraging honey bees. With climate models predicting increases in wind speeds, understanding how we can help pollinators in a changing climate is becoming ever more pressing.

Chapter Six: Wind slows play: Increasing wind speed reduces flower visiting rate in honeybees

6.1 Abstract

Wind is an understudied environmental variable capable of having profound impacts upon the foraging behaviour of flying organisms. We investigated the effects of wind and temperature on *Apis mellifera* foraging on two plant varieties, *Lavandula x intermedia* 'Grosso' and *Origanum vulgare* to determine how wind influences foraging behaviour and if responses differ between the plant varieties. Fans and an artificial wind break were used to generate wind speeds from 0-3.5m/s with the following behaviours examined in a minute of foraging: number of flower visits, handling time per flower, inter-flower flight duration and proportion of flowers walked to. Flower movement was quantified using video footage and imageJ tracking software. Bees visited significantly fewer flowers with increasing wind speed on both plant varieties, with an average decrease of 38% between the lowest and highest windspeeds. However, the reduction was significantly steeper when foraging on *O. vulgare*. The reduction was due to an increase in handling time on both varieties, with inter-flower flight duration unaffected. Temperature had no effect on flower visit rate for either plant variety. The only behaviour influenced by flower movement was handling time, which was found to increase with flower movement. However, this increase did not result in fewer flower visits. Our results support previous work on artificial flowers which determined that the direct effect of wind on the bee influences foraging efficiency and behaviour more than the indirect effect of flower movement.

6.2 Introduction

Animals face many challenges when foraging, including the effects of variation in their immediate environment (Porter and Tschinkel 1987, Soulsbury et al 2008), which can cause dramatic shifts in behaviour. For example, bald eagles do not forage in high winds and rain (Elliott et al 2006) and Atlantic salmon will switch from foraging during the day to the night in response to cold temperatures (Fraser et al 1995).

Wind is an important environmental factor that is capable of influencing foraging behaviour, both positively and negatively. Shearwaters take advantage of strong winds to travel to feeding locations further away from the nest (De Pascalis et al 2020) whereas high winds cause leaf cutter ants to collect smaller sized pieces of vegetation when foraging, reducing the amount of food a colony is able to collect (Alma et al 2017). Wind poses significant challenges for many flying animals, especially insects, who regularly interact with the unsteady airflow and turbulence created by wind (Combes and Dudley 2009) and whose small body size, resulting in a larger surface area to volume ratio, will make increasing wind speed a greater challenge than for birds, due to the increased effects of air resistance (Hunter 2007). Wind has been shown to affect the foraging activity of several insect species. For example, the number of honeybees foraging on watermelon and apple trees decreased in increasing wind speed (Pinzauti 1986, Vicens and Bosch 2000), and bumblebees flew longer distances downwind than upwind on windy days (Comba 1999). When flying in wind vs still air, energy expenditure for euglossine bees increased by 30% due to increased drag resulting from the lowering of the hindlegs for stability (Combes and Dudley 2009). For honey bees foraging on artificial flowers, increasing wind speed was found to cause an increase in take-off hesitation (Hennessy et al 2020).

The honey bee, *Apis mellifera,* has been estimated to make between 250 and 1446 flower visits per trip (Ribbands 1949, Goodwin et al 2011), many of these requiring a bee to fly between individual flowers or even plants. *A. mellifera* use a combination of passive and active changes to body positioning in response to wind, and alter wing beat frequency to stabilise body positioning to produce efficient flight (Vance et al 2013, Ravi et al 2016, Crall et al 2017). However, it is not only the flight between flowers that can be influenced by wind. Flowers themselves sway in windy conditions, potentially making take-off and landing harder. A recent experimental study of *A. mellifera* foraging on artificial flowers in increasing winds found that a 2.75m/s increase in wind speed caused a 37% reduction in the mean

number of flowers visited when flowers were kept stationary. However, when the artificial flowers were moved under zero wind conditions, there was no change in flower visiting rates (Hennessy et al 2020). However, this study did not expose insects to the combined effects of wind and flower movement.

Plant species themselves have adaptations to aid pollinators foraging in windy conditions. Some have flowers with conical shaped cells on their surface that assist flower handling through improved grip (Whitney et al 2009), which is potentially beneficial to pollinators in windy conditions when the flower itself is moving (Alcorn et al 2012). However, flower movement may also be beneficial to plants. Warren and James (2008) found that flowers which had moderate mobility attracted more pollinators than those which were stationary. (Wolf & Zerrahn-Wolf 1937) found similar results, with *A. mellifera* choosing to forage on moving versus immobile artificial flowers. Flowers exhibit a wide range of morphologies which are expected to interact differently with wind and pollinators. For example, larger flowers may be easier to see and land on and flowers placed on long inflorescences are likely to sway more in windier conditions (Warren and James 2008). Flower morphology is capable of influencing handling time in the absence of wind (Balfour et al 2013). However, no studies have examined differences in foraging efficiency on different plant species in the presence of wind.

The aim of this project was to investigate the effects of wind speed, temperature and flower movement on *A. mellifera* foraging behaviour on two different plants. We studied free flying honey bees in the laboratory garden as they foraged on hybrid lavender (*Lavandula x intermedia* 'Grosso') and wild marjoram (*Origanum vulgare*).

6.3 Methods

6.3.1 Flower patches and wind speeds

We studied free flying *Apis mellifera* foraging on *Lavandula x intermedia* 'Grosso' (lavender) and *Origanum vulgare* (marjoram) plants in bloom on the campus of the University of Sussex. Data collection took place on 6 days between 5 and 27 July, 2018. Two 160x60cm patches of each plant variety within a larger bed of that variety were marked out using bamboo canes, each located in the same garden and approximately 6m apart. We used a combination of three fans (Olypa H-28401 Electrical 16-Inch Oscillating Pedestal Stand fan)

and a wind break made from bamboo canes supporting plastic mesh to cause winds ranging from 0-3.5 m/s. The fans were placed beside the patch and arranged in such a way as to ensure near equal windspeed throughout the patch at any particular time. To determine the mean wind speed for each bee's trial a digital omnidirectional hand held anemometer (Skywatch Eole, sensitivity 0.1m/s) was placed at the centre of each patch and videoed for the entire minute of a trial. The video was played back on a computer and the wind speed was noted every 10 seconds. The average was used as the wind speed experienced in that trial. By adjusting the fans, we generated a random sequence of wind speeds in three categories: 0-1.5, 1.5-2.5, 2.5-3.5 m/s. These categories were chosen in part as they were the ranges of wind speed obtained by using the available fan settings. In addition, they were realistic in relation to common wind speeds in the area, but which were not too high to prevent bee activity (Khurana & Sane 2016). Temperature was recorded from the centre of the patch at the end of each bee's foraging trial using a digital thermometer (KTJ^{TM}) and was recorded to the nearest degree.

6.3.2 Observing foraging bees in patches

A trial consisted of observing, by eye, a foraging bee that was in the patch for one minute, recording the following behaviours:

I) Number of flower visits: Number of flowers where a bee landed and was seen to probe to take nectar. If multiple flowers were visited on the same inflorescence they were treated as separate flower visits.

II) Mean flower handing time: The total time spent on flowers divided by the number of flower visits within the minute of observation. This included time when bees walked between flowers, but did not include when they were in flight, and was recorded by stopwatch.

III) Mean duration of inter-flower flights: The total time spent flying between flowers divided by the number of individual flights. Time spent flying was also recorded by stopwatch.

IV) Proportion of flowers walked to: Number of times a bee walked to a flower divided by total flower visits via walking plus flying.

After one minute, the bee was caught and marked with a paint pen to ensure that previously tested bees were not retested. All time recordings were measured to the nearest 0.01s.

6.3.3 Flower movement

To quantify flower movement in increasing wind speeds, lavender and marjoram flowers were videoed with a Sony camera (HDR-CX115) on a tripod at average flower height and 2m away from a reference point within the patch, which was marked with a bamboo cane (Fig 1). An anemometer was placed within the patch to record wind speed and a fan was placed diagonally facing the flowers. Videos, 24 frames per second, were made at a range of wind speeds (0-2.3m/s) and were imported into the software imageJ and individual flowers were chosen at random from the reference point and their location tracked in each frame for 100 frames (4 seconds). For each frame the wind speed was also recorded. The average wind speed and flower movement (cm/s) were calculated for 30 flowers per plant variety. Because movement was observable in 2-dimensions only, longitudinal movement was not quantifiable. This method has been used in previous work assessing leaf movement (Kothari and Burnett 2017).

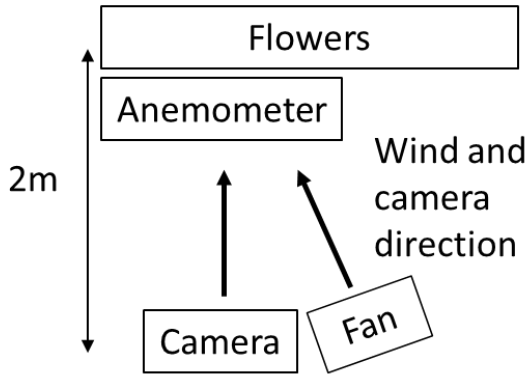


Fig 1: A top down view of the experimental set up for recording the flower movement of lavender and marjoram plants. The camera was always placed 2m from a reference point within the patch of flowers and the anemometer was placed immediately in front of the flowers being recorded to ensure the wind speed those specific flowers were experiencing was measured. Figure is not to scale.

6.3.4 Statistical analysis

Statistical tests were performed in R 3.3.3 (R Development Core Team 2009). Linear mixed effects models were run for each of the observed behaviours with flower patch included as a random effect. Models were compared using AIC values and tested against model assumptions using the DHARMa package (Hartig 2020). The predictor variables in each model included average wind speed, temperature, plant variety and average flower movement. Interactions between plant variety and both wind speed and temperature were also included. Average flower movement for each plant variety was calculated from three wind speed categories, 0-1m/s, 1.1-2m/s, and >2m/s. Due to multicollinearity between average wind speed and average flower movement all predictor variables were standardised through centering. This was achieved by subtracting each predictor variable's mean from all observations on that variable, meaning the new mean for each predictor variable is zero. (Iacobucci et al 2015).

subtracting a variable's mean from all observations on that variable in the dataset such that the variable's new mean is zero Handling time and duration of inter-flower flight were log transformed to obtain normality and a generalised linear mixed effects model with a binomial distribution was used when analysing proportion of flowers walked to. A three-way interaction between wind speed, temperature and plant variety was tested but was not significant for any behaviour and was therefore removed from the analyses. The interaction between wind speed and temperature was also non-significant for either plant species and was therefore removed. The interaction between average flower movement and wind speed was tested. However, it was never significant and resulted in high variance of inflations factors (VIF >10) so was therefore removed from models. A Spearman's rank correlation was run on average wind speed and temperature to determine if the two variables were correlated. The final model for each of the behaviours was:

Behaviour~ Average wind speed*plant variety+ temperature*plant variety +Average flower movement+(1| patch)

To test the influence of wind speed on flower movement, a linear regression was run with average flower movement as the dependent variable and average wind speed and plant variety as the predictor variables.

6.4 Results

We studied a total of 350 free flying *A. mellifera,* 190 on lavender and 160 on marjoram. Wind speeds ranged from 0-3.5m/s and temperatures from 19 to 28°C. Temperature and wind speed were not correlated (r_s= -0.023, P=0.670).

6.4.1 Number of flower visits

The number of flower visits for *A. mellifera* foraging on both lavender and marjoram significantly reduced with increasing wind speed ($F_{1,344}$=38.51, P<0.001, Fig 2a). There was a 36% decrease from lowest (0-1 m/s) to the highest (2.5 -3.5m/s) wind speeds for lavender, and a 41% decrease on marjoram (Table 1). Flower movement has no effect on number of flower visits ($F_{1,344}$=2.68, P=0.102). Temperature had no effect on either lavender or marjoram ($F_{1,344}$=0.29, P=0.585, Fig 2b). The interaction between plant variety and wind speed was significant ($F_{1,344}$=12.75, P<0.001) with a greater decrease in flower visits with increasing wind speed on marjoram (Table 2). Overall, across wind speeds, bees visited significantly fewer lavender than marjoram flowers ($F_{1,344}$=23.26, P=0.034). The interaction between plant variety and temperature was not significant ($F_{1,344}$=0.04, P=0.847).

6.4.2 Mean flower handling time

The mean flower handling time significantly increased with wind speed when *A. mellifera* were foraging on either flower variety ($F_{1,344}$=28.87, P<0.001, Fig 2c). Between low and high wind speeds when foraging on lavender, handling time increased by 35%, from 2.68±0.15s to 3.82±0.16s. For marjoram, handling time increased by 29%, from 1.80 ± 0.12s to 2.40 ±0.20s at the highest wind speeds.

There was a significant difference in handling time between plant varieties independent of wind speed ($F_{1,344}$=22.55, P=0.040) with *A. mellifera* taking longer on average per flower on lavender than marjoram (3.35s ± 0.08s vs 2.07s ± 0.01). Flower movement significantly increased handling time on both lavender and marjoram flowers ($F_{1,344}$ =4.02, P=0.046, Fig 3). However, this had less of an effect on handling time compared to wind speed for both plants (Table 2). Temperature alone and both the two-way interactions between plant variety and wind speed and plant variety and temperature were not significant (temperature; $F_{1,344}$=0.002, P=0.968, plant species:*wind speed; $F_{1,344}$=2.17, P=0.141, plant species*temperature; $F_{1,344}$=0.489, P=0.484, Table 2, Fig 2d).

6.4.3 Mean duration of inter-flower flight

Perhaps surprisingly, wind speed had no effect on the duration of inter-flower flights on either plant variety ($F_{1,344}$=1.22, P=0.270, Fig 2e, Table 1). Flower movement was also not significant ($F_{1,344}$=0.55, P=0.458) The interaction between temperature and plant variety was significant ($F_{1,344}$=6.51, P=0.011). Temperature alone had a significant effect when *A. mellifera* were foraging on lavender (t=3.05, P=0.003), with mean flight duration increasing with temperature, but had no effect when foraging on marjoram (t=-0.50, P=0.617, Fig 2f). The interaction between wind speed and plant variety was not significant ($F_{1,344}$=0.955, P=0.329, Table 2).

6.4.4 Proportion of flowers walked to

Increasing wind speed had no effect on proportion of flowers walked to on either lavender or marjoram (lavender; Z=0.957, P=0.957, marjoram; Z=-1.32, P=0.186, Fig 2g). *A. mellifera* walked to a significantly smaller proportion of flowers when foraging on lavender compared to marjoram independent of wind speed and temperature (Z=3.49, P<0.001). No other variables significantly influenced proportion of flowers walked to when *A. mellifera* were foraging on either marjoram or lavender (Table 2).

Table 1**:** The mean responses of all studied foraging behaviours on both plant species at the different levels of wind speed. Low wind was classed as speeds between 0-1m/s with high classed as 2.5-3.5m/s. Standard errors are given along with the number of honey bees (*N*) in each category for each plant variety.

	Lavandula x intermedia 'Grosso'		*Origanum vulgare*	
Behaviour	Low wind speeds 0-1m/s *N*=59	High wind speeds 2.5-3.5m/s *N*=33	Low wind speeds 0-1m/s *N*=66	High wind speeds 2.5-3.5m/s *N*=26
Number of flower visits	20.53±0.94	14.3 ±1.65	32.03 ±1.14	24.1 ±1.77
Mean flower handling time (s)	2.68 ±0.15	3.82 ±0.20	1.80 ±0.12	2.40 ±0.20
Mean duration of inter-flower flight (s)	1.14 ±0.12	1.19 ±0.10	0.72 ±0.09	0.62 ±0.11
Proportion of flowers walked to	0.68 ±0.02	0.55 ±0.03	0.80 ±0.02	0.80 ±0.04

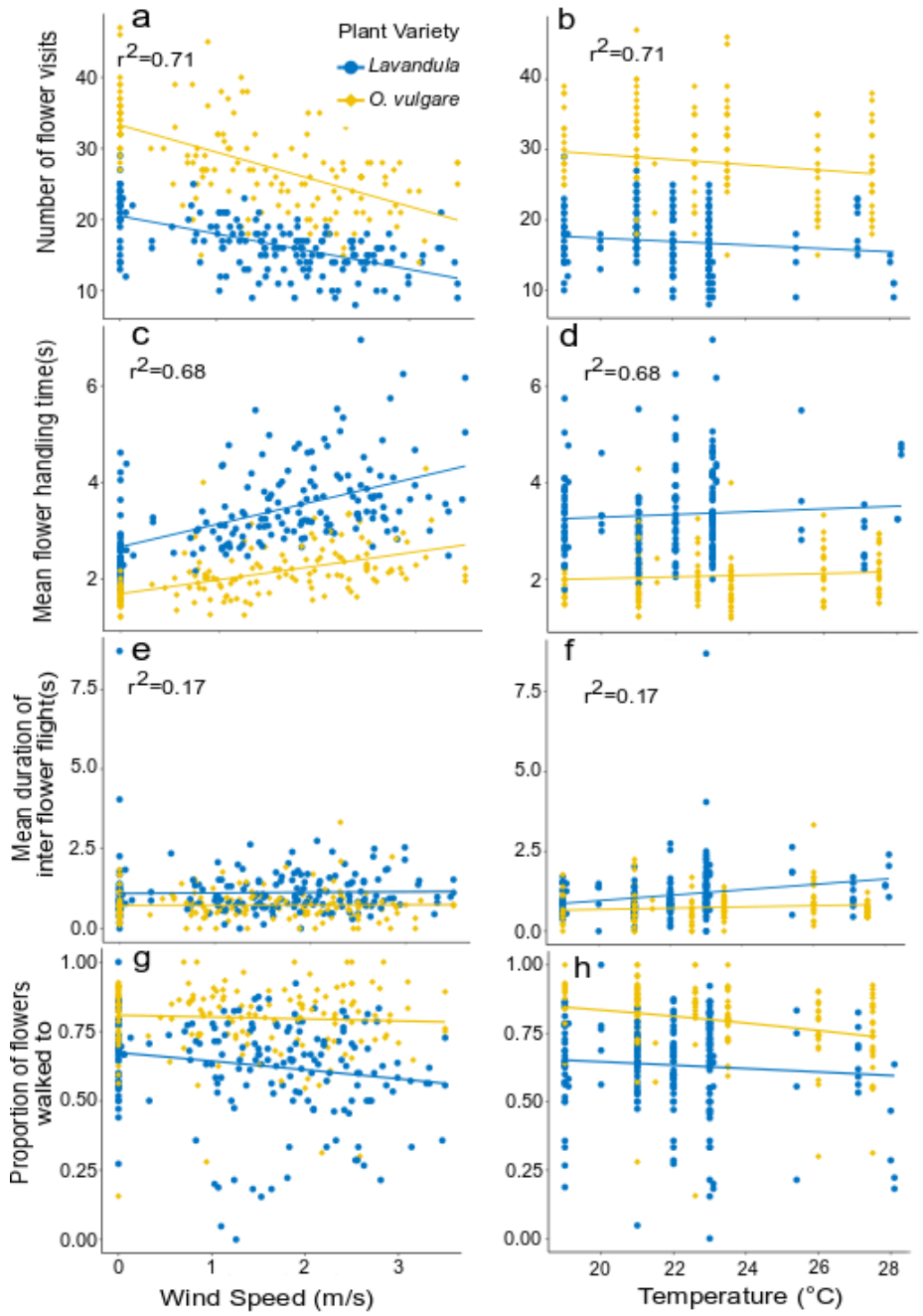


Fig 2: Bee foraging behaviours of *A. mellifera* workers foraging for one minute on either *Lavandula x intermedia 'Grosso'* or *Origanum vulgare.* (a-b) Number of flower visits in relation to (a): wind speed; (b): temperature; (c-d) mean flower handling time in relation to (c): wind speed and (d): temperature, (e-f) the mean duration of flight bout in relation to wind speed and (f): temperature, (g-h) proportion of flowers walked to in relation to (g): wind speed and (h): temperature. Linear regression lines are shown for a-f and a binomial regression line is shown for g and h. R^2 values are given for the behaviours tested with linear regression models. *N*=190 for lavender and *N*=160 for marjoram for all behaviours.

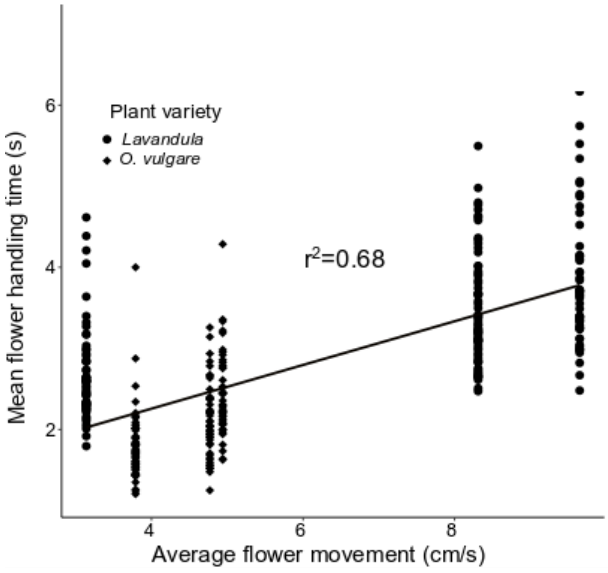


Fig 3: Average flower handling time for *Apis mellifera* in relation to average flower movement when foraging on either *Lavandula x intermedia 'Grosso'* (*N*=190) and *Origanum vulgare* (*N*=160).

Table 2: Final model summary outputs of LMMs, linear mixed effects models for all tested behaviours. The effects of wind speed, plant variety, temperature and flower movement and the interactions between temperature and plant variety and wind speed and plant variety on number of flower visits, mean handling time (log), mean duration of inter flower flight (log) and proportion of flowers walked to (where a GLMM with a binomial error structure was run and the Z value is given). Bold indicates a significant effect on the response variable. The results for both *Lavandua x intermedia* and *Origanum vulgare* are shown.

		***Lavandula x intermedia* 'Grosso'**				***Origanum vulgare***			
Flower visits	Fixed effects	Estimate	SE	t	P	Estimate	SE	t	P
	Intercept	16.22				35.42			
	Average wind speed	**-1.43**	**0.67**	**-2.14**	**0.033**	**-3.76**	**0.33**	**-11.28**	**<0.001**
	Plant variety	**11.43**	**2.37**	**4.82**	**0.034**	**-11.43**	**2.37**	**-4.82**	**0.034**
	Temperature	-0.04	0.15	-0.24	0.808	-0.077	0.15	-0.54	0.592
	Average wind speed * variety	**-2.33**	**0.65**	**-3.57**	**<0.001**	**2.33**	**0.65**	**3.57**	**<0.001**
	Temperature* plant variety	-0.40	0.21	-0.19	0.846	0.040	0.21	0.19	0.846
	Flower movement	-0.41	0.25	-1.63	0.102	-0.41	0.25	-1.64	0.102
Mean handling time	Intercept	1.21				0.73			
	Average wind speed	**0.08**	**0.03**	**2.63**	**0.009**	**0.13**	**0.02**	**8.19**	**<0.001**
	Plant variety	**-0.47**	**0.10**	**-4.75**	**0.040**	**0.47**	**0.10**	**4.75**	**0.040**
	Temperature	-0.00	0.01	-0.51	0.610	0.00	0.00	0.48	0.633
	Average wind speed *plant variety	0.05	0.03	1.47	0.141	0.05	0.03	-1.47	0.141
	Temperature*plant variety	0.01	0.01	0.70	0.485	-0.01	0.01	-0.70	0.485
	Flower movement	**0.02**	**0.01**	**2.00**	**0.46**	**0.02**	**0.11**	**2.00**	**0.046**
Mean duration of inter flower flight	Intercept	0.77				0.49			
	Average wind speed	0.05	0.04	1.16	0.246	0.01	0.02	0.43	0.665
	Plant variety	-0.28	0.11	-2.40	0.114	0.28	0.11	2.40	0.114
	Temperature	**0.03**	**0.01**	**3.05**	**0.003**	-0.005	0.01	-0.50	0.617
	Average wind speed *plant variety	-0.04	0.04	-0.98	0.329	0.04	0.04	0.98	0.329
	Temperature* plant variety	**-0.03**	**0.01**	**-2.55**	**0.011**	**0.03**	**0.01**	**2.55**	**0.011**
	Flower movement	-0.01	0.02	-0.74	0.458	-0.01	0.02	-0.74	0.458
				Z				Z	
Prop. flowers walked to	Intercept	0.63				2.06	1.36		
	Average wind speed	-0.01	0.09	-0.05	0.957	-0.08	-0.05	-1.32	0.186
	Plant variety	**0.96**	**0.27**	**3.49**	**<0.001**	**-0.96**	**0.27**	**-3.49**	**<0.001**
	Temperature	-0.01	0.02	-0.26	0.796	-0.02	0.02	-1.35	0.177
	Average wind speed *plant variety	0.05	0.05	0.96	0.337	0.05	0.08	0.59	0.558
	Temperature* plant variety	-0.02	0.03	-0.74	0.458	0.02	0.03	0.72	0.471
	Flower movement	-0.05	0.03	-1.53	0.126	-0.05	0.03	-1.53	0.126

6.4.5 Flower movement

Flower movement significantly increased with wind speed for both plant varieties (linear regression, $F_{1,58}$=46.52, P<0.001, Fig 4). The interaction between variety and wind speed was also significant ($F_{1,58}$=5.25, P=0.026), with lavender flowers having a greater increase in average movement with wind speed (interaction coefficient estimate: 2.15). Movement also differed significantly between varieties ($F_{1,58}$=14.70, P<0.001). At the lowest wind speeds (0-1m/s) lavender flowers had an average speed of 3.18 ±0.14 cm/s and marjoram of 3.79 ±0.14 cm/s. At higher wind speeds (1.5-2.3m/s) lavender flower movement increased by 100%, to 9.51 ±0.12 cm/s and for marjoram the increase was 40%, to 5.69 ±0.09 cm/s.

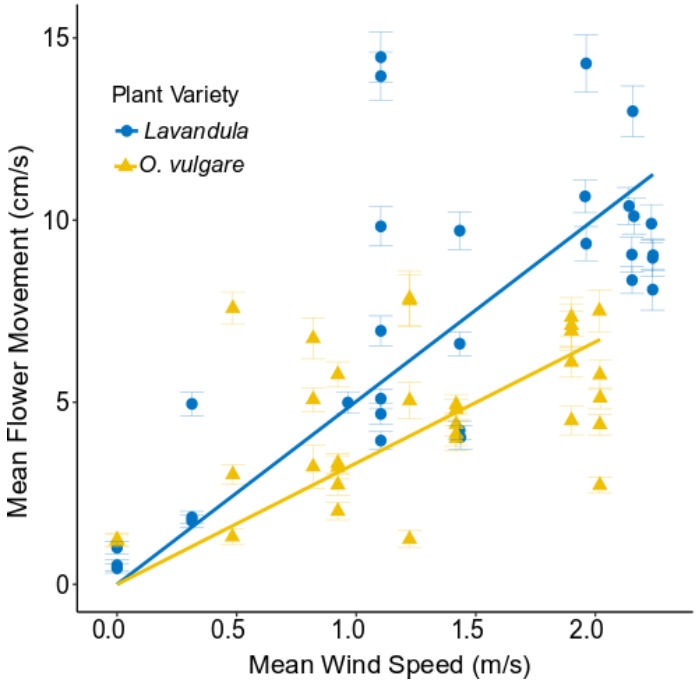


Fig 4: Mean speed (cm/s) of *Lavandula x intermedia* 'Grosso' and *Origanum vulgare* flowers in relation to increasing wind speed. Each point is the average flower movement and wind speed taken from 100 frames (4 seconds) of video analysed using ImageJ manual tracking plugin. Regression lines are shown.

6.5 Discussion

The results show that *A. mellifera* foraging behaviour and efficiency is greatly influenced by wind on both lavender and marjoram flowers. In particular, the number of flower visits per minute, which is a key measure of foraging performance, significantly

decreased with increasing wind speed, with a greater decrease when bees were foraging on marjoram (Fig 1a). This decrease in number of flower visits was due to an increase in flower handling time on both flower varieties (Fig 1b). Flower movement did not influence flower visit rate. However, it did significantly increase handling time (Fig 3).

There was a significant difference between plant varieties in the reduction in flower visits with increasing wind speed, with a greater reduction on marjoram. There was also a difference in flower movement speeds in relation to wind between the two plant varieties. However, the difference in flower visits does not appear to be due to flower movement, with both flower movement alone and its interaction with wind speed not significantly influencing the number of flower visits. This agrees with research on honey bees foraging on artificial flowers, where wind speed alone caused a reduction in flower visits, but flower movement alone did not (Hennessy et al 2020).

Although flower movement did not influence flower visit rate, it did cause an increase in average flower handling time. However, this increase was not large enough to reduce visit rate independently of wind speed. Our results cannot indicate what stage of the flower handling process is being influenced by flower movement, as it encompassed all the behaviours when a bee was on a flower. These include walking between flowers, probing for nectar and take off hesitancy. However, previous work on *Bombus terrestris* found that foraging bees will choose flowers with better grip (those that possess conical shaped cells on their petals), versus those with smoother petals when flowers were moving, due to the increased difficulties of handling flowers (Alcorn et al 2012). Lavender flowers moved significantly more with increasing wind speed compared to marjoram flowers, perhaps due to flowers being on longer stalks (Warren and James 2008). The fact that flower visit rate reduced significantly more on the less mobile marjoram flowers, and that flower movement was not found to reduce visit rate, suggests perhaps a combination of differences in flower morphology and wind speed are responsible for flower visit reduction seen in increasing winds.

Slight caution should be taken when interpreting the interaction between flower movement and wind speed. Flower movement and wind speed are expected to be highly correlated, meaning that on slow moving flowers bees will mainly be subject to low wind speeds, and vice versa. To fully understand the interaction between wind speed and flower movement and

its effects on honeybee foraging behaviour, manipulation experiments may be required to separate the two effects.

Flower handling time increased with wind speed on both plant varieties. This result is consistent with previous work (Hennessy et al 2020) which found that *A. mellifera* foraging on artificial flowers visited fewer flowers per minute with increasing wind speeds due to an increase in hesitancy to take off from a flower at the end of the visit. In the current study, hesitancy was not directly quantified, but would have been included in the flower handling time. Although we cannot be sure, it is likely that increased take off hesitancy occurred in this study, with bees perhaps spending longer readying themselves for take-off at greater wind speeds. In cluttered environments, such as amongst vegetation, the air is often unstable, with the presence of vorticial wakes, such as von Karman streets, thereby presenting flight control challenges for insects (Jakobi et al 2018, Barnett et al 2020). The position of the body prior to take-off is important, as certain body angles affect stability (Vance 2013). Obtaining an optimal position before take-off could reduce the in- flight requirements for stability, but with a longer time cost when on the flower to reach the correct body position. Previous work on cereal aphids found that individuals increase their take off hesitancy in increasing wind speeds (Walters et al 1984) and the damselfly *Enallagma doubledayi* was found to orientate itself towards the wind when taking off (Mason 2017). Both these studies show that when wind is present there are adaptive shifts in behaviour during the take-off phase that are likely increasing take-off hesitancy.

Perhaps surprisingly, both wind speed and flower movement had no effect on inter-flower flight duration. In previous studies wind has been found to influence various aspects of both bee flight mechanics and behaviour to compensate for the effects of wind (Riley et al 1999, Combes and Dudely 2009, Crall et al 2015, Crall et al 2017). For example, when landing in still air bumblebees will decelerate their flight speed on approach to a flower. However, when landing in wind their flight speed does not reduce on approach, resulting in a ‘crash landing’ (Chang et al 2016). Burnett et al (2020) found that when navigating moving obstacles in still air, *A. mellifera* had slower flight speeds compared to when navigating obstacles in headwinds or tailwinds. These alterations to flight speed when flying in wind may help explain why flight bout duration was unaffected, as bees perhaps increase their flight speeds in response to increasing wind speed.

Flower movement also had no effect on flight-bout duration. In previous studies examining flower movement, moderately wavy flowers were found to attract more pollinators, including honeybees, versus stationary or highly mobile flowers (Warren and James 2008). This increased attraction may be due to moving flowers being easier for insects to detect visually. Bees are unable to use stereoscopic vision, and instead rely on motion parallax for depth perception (Lehrer 1998). This means that moving objects against a stationary background are easier to see than still objects (Alcorn et al 2012, Kapustjansky et al 2010). In field experiments *A. mellifera* chose moving versus stationary flowers (Wolf & Zerrahn-Wolf 1937) and are capable of landing accurately on moving objects by correcting for angular deviations on approach (Zhang et al 1990, Burnett et al 2020). We hypothesise that perhaps bees increase their flight speed in response to increasing wind, and although this increase in speed may make controlled landings more difficult (Chang et al 2016), flower movement helps negate these difficulties by increasing the ease of seeing and landing on a flower (Kapustjansky et al 2010). These factors combined may result in no change in flight duration when foraging in increasing wind speeds.

The proportion of flowers bees walked to was also unaffected both by wind speed and flower movement. *A. mellifera* have highly complex tarsi, giving them a strong grip even on smooth surfaces (Brauer 2017). Perhaps this strong grip ensured that neither flower movement nor wind speed influenced bees' abilities to walk between flowers. Future studies could assess the impact of wind on bees' abilities to travel between flowers further by using slow motion videos of foraging bees, breaking down the stages of flight to determine how inter-flower flight, landing and walking between flowers are influenced by both wind and flower movement. Future work could also assess the potential increase in metabolic costs associated with foraging in high winds, and the implications this may have on foraging behaviour.

The only behaviour which temperature was found to influence was the duration of inter flower flight, with the effect dependent on plant variety. Flight duration increased with increasing temperature when honeybees were foraging on lavender but there was no significant correlation when foraging on marjoram. Why this difference exists we cannot explain with our data. There are several variables not measured in this study that vary with temperature, and these may influence honeybee flight duration. For example, nectar availability and its distribution throughout a patch can be indirectly influenced by temperature through increased competition, due to higher numbers of competitor foragers in better weather conditions (Comba 1999). Although not directly measured in this study, there

were other species also foraging on the patches being observed. Indirect competition from these other species, especially bumblebees, may cause foraging honeybees to have to fly for longer to locate a flower containing nectar (Balfour et al 2015), with increasing temperatures perhaps increasing the potential for competition. Another variable not accounted for in this study was floral bloom per patch, which is capable of influencing flower visit rate (Klinkhamer et al 1989, Ohashi and Yahara 2002). However, data was collected on two patches for each plant variety of the same size across the same period, which would reduce any differences in the number of flower units present per patch.

This study has highlighted that even on plant varieties in the same family, *A. mellifera* foraging behaviour in response to wind, a highly variable and relevant environmental factor, can be different and that wind can have major and complex effects on honeybee foraging behaviour and performance. Increasing wind is capable of significantly reducing foraging efficiency, with the rate of reduction influenced by the flower species. This reduction appears to be primarily due to the effects of wind on the bee, and less so by the effects of the wind on the movement of flowers. However, the exact reason for the reduction is unknown. Future work could examine in more detail the entire inter-flower flight process and examine separately take-off, flight between flowers and landing, identifying which behaviour is being most influenced by wind. Also, by studying a greater variety of plant species there can be a better understanding of why differences in foraging response is seen between species. Although bees foraging on flowers is one of the most easily seen of all behaviours and wind is an ever-changing environmental factor that bees deal with all the time, the interaction between bees, flowers, and the wind is surprisingly complex.

Chapter Seven: Blowin' in the wind: Honeybee but not bumblebee flower visiting rate is significantly reduced with increasing wind when foraging on lavender (*Lavandula spp.*)

7.1 Abstract

Wind is an understudied environmental variable factor that affects bee behaviour. We examined the effects of increasing wind speed on *Apis mellifera* and *Bombus* species foraging behaviour on lavender (*Lavandula x intermedia* 'Grosso') and compared differences in species' response. Fans and an artificial wind break were used to generate wind speeds of 0 to 3.5ms^{-2} with the following behaviours examined in one minute foraging trials on individual bees: number of flower visits, handling time per flower, inter-flower flight duration and proportion of flowers walked to. The number of *Bombus* foragers and the time individual foragers spent within a patch with and without wind were determined. *A. mellifera* flower visits significantly decreased with increasing wind speed by 36% from the lowest to highest wind speeds due to increasing handling time per flower. However, *Bombus* flower visiting rate and handling time per flower were unaffected. The number of *Bombus* foragers on a patch and the time spent within the patch significantly decreased with wind speed. There were significantly more *Bombus* foragers on neighbouring patches without wind. Our results suggest that *Bombus* species are less affected by wind than honey bees when foraging on lavender. Future work examining the responses of pollinator species to wind on a variety of plant species is needed to fully understand the causes of these differences and their potential implications in nature.

7.2 Introduction

Foraging takes place in a variable environment, with abiotic factors such as temperature, rain and light constantly changing and potentially affecting and causes changes in foraging behaviour. For example, the Western Australian magpie reduces foraging effort at temperatures above 27°C and instead invests time in heat dissipation behaviours (Edwards et al 2015). Rain can cause bats to reduce foraging activity due to an increase in energy expenditure when flying with wet wings (Voigt et al 2011).

Wind is an environmental variable which can affect both forager behaviour (Comba 1999, Alma et al 2016, Berlincourt et al 2015, Hennessy et al 2020) and forage abundance and accessibility (Møller 2013 and Teglhøj 2017). For example, leaf cutter ants reduce their walking speed by 55% and are blown off course 28 times more often when foraging in windy compared to still conditions (Alma et al 2016). Red deer will forage on lower quality resources if located in an area which provides shelter from the wind (Conradt et al 2000).

The influence of wind on birds, both in relation to migration and foraging, has been quantified for many species (Åkesson and Hedenstrom 2000, Erni et al 2002, De Pascalis 2020). However, less is known about the effects of wind on flying insect foraging behaviour, even though wind should be especially challenging to insects due to their small body size, leading to a larger surface area to volume ratio. We would expect that the small size of insects will affect their flying ability not just via effects of head, side or tail winds, but also by increasing any effects due to unsteady airflow, including buffeting and turbulence (Combes and Dudley 2009). Studies on wind and flying insects have often focused on how it effects flight mechanics (Crall et al 2017, Ravi et al 2013) or how it reduces foraging activity (Pinzauti 1986, Vicens and Bosch 2000). One study, which examined the effects of increasing wind on specific foraging behaviours of *Apis mellifera* found that the flower visiting rates decreased with increasing wind speed due to an increase in the hesitancy to take-off from a flower (Hennessy et al 2020). However, this study was on artificial flowers and did not examine the interaction between flower movement and wind speed.

It is often noted that the pollinator community changes depending on weather conditions (Tuell and Isaacs 2010, Vicens and Bosch 2000, Brittain et al 2013). Honeybees, *Apis mellifera*, are regarded as the dominant pollinators of many of our food crops and some flowers (Rader et al 2013). However, they tend not to forage below 10˚C and in windy conditions (Pinzauti 1986). This can create a pollination deficit for certain plant species

during times of bad weather (Vicens and Bosch 2000). Bumblebee species, on the other hand, are capable of foraging at lower temperatures (e.g. 3°C, Steltzer & Chittka 2010) and are often seen foraging in wind (Vicens and Bosch 2000, Brittain et al 2013). This may be due to them possessing thermoregulatory abilities which allow them to maintain a thoracic temperature many degrees above ambient temperature when in flight (Heinrich 1975). Bumblebees have been found to increase their approach speed when landing in windy conditions (Chang et al 2016) and will make longer downwind flights on windy days (Comba 1999). However, how these shifts in behaviour influence flower visits on real flowers is not known.

The aim of this project was to investigate and compare the effects of wind speed and temperature on *A. mellifera* and *Bombus terrestris/lucorum* foraging behaviour when foraging on *Lavandula x intermedia* 'Grosso'. As well as studying the species behavioural responses, the number of *Bombus* foragers in patches of *Lavandula x intermedia* 'Grosso' with and without wind, along with how long foragers spent within these patches was also determined.

7.3 Methods

We studied free flying *Apis mellifera* and *Bombus terrestris/lucorum* foraging on *Lavandula x intermedia* 'Grosso' (lavender) plants in bloom on the campus of the University of Sussex. Data collection took place on 17 days between July 5 and 27, 2018. Three 160x60cm patches of lavender within a larger bed (10x10m) were marked out using bamboo canes. Using a combination of three fans (Olypa H-28401 Electrical 16-Inch Oscillating Pedestal Stand fan) and a wind break made from bamboo poles and plastic mesh we produced winds ranging from 0-3.5 ms^{-1}. The fans were positioned to cause a near equal distribution of wind speed throughout each patch. To determine the wind experienced by each study bee, during each trial, a digital omnidirectional anemometer (Skywatch Eole) was placed at the centre of each patch and videoed for the entire minute of bee observation. The video was played back on a computer and the wind speed every 10 seconds was noted. The average speed was then used as the wind speed experienced by the bee being studied in that trail, with wind speed recorded to the nearest 0.01 second. Wind speeds were randomly selected within three categories: 0-1.5, 1.5-2.5, 2.5-3.5 ms^{-1}. Temperature was also recorded at the centre of the patch at the end of each trial using a digital thermometer (KTJ^{TM}) to the nearest 0.01 degree.

7.3.1 Observations of foraging bees in patches of flowering lavender

A trial began when a bee flew into a study patch and was then observed for one minute to record the following behaviours:

I) Number of flower visits: Number of flowers where a bee was seen to take nectar or collecting pollen. A flower was recorded both when flown to or when walked to. If a bee visited multiple flowers on the same inflorescence each was considered a visit.

II) Mean flower handing time: The total time spent on flowers divided by the number of flower visits within the minute of observation. Time was recorded using a stop watch.

III) Mean inter-flower flight time: The total time spent flying divided by the number of individual flights. Time was also recorded using a stopwatch.

IV) Proportion of flowers walked to: Number of times a bee walked to a flower divided by total flower visits

When the subject bee had been observed for one minute she was caught and marked with a paint dot to ensure the same bee was not studied twice. In addition, *B. terrestris/lucorum* bees were weighed as they can vary in body size. *A. mellifera* were not weighed as workers are similar in size. Due to difficulties in catching individual bees, not all were successfully weighed or marked.

7.3.2 Number of foragers in patches

To identify the relationship between increasing wind speed and forager activity on lavender, three 120x90cm patches of lavender in an existing bed (10x10m) were marked, 120cm apart, using bamboo canes. Three fans (Olypa H-28401 Electrical 16-Inch Oscillating Pedestal Stand fan) were placed surrounding one of the patches to produce wind, and a digital omnidirectional anemometer (Skywatch Eole) and digital thermometer (KTJ^{TM}) were placed at the centre of the patch to record wind speed and temperature. One patch never experienced artificial wind. The other two patches were alternated between artificial wind or no artificial wind every half hour. Counts occurred every 10 minutes, with the number of *Bombus* foragers recorded along with the wind speed and temperature within each patch. A total of 59 counts were made over three days, from August 1 to August 3, 2018. *A. mellifera* were not included as few were present. Although counted separately, *Bombus* species were pooled for analysis due to low numbers of some species.

7.3.3 Time spent within a patch

Using the same lavender patches for forager counts the time a forager spent within a patch with and without wind was recorded. A bee was chosen at random when she entered an experimental patch. She would be timed using a stopwatch from when she landed on a flower to when she exited the patch (which was defined as when she had flown outside of the bamboo perimeter). Data were collected from 70 *Bombus* foragers. Again, due to the small numbers of some *Bombus* species, data were pooled for analysis.

7.3.4 Statistical analysis

Statistical tests were performed in R 3.3.3 (R Development Core Team 2009). Linear regression models were run for each of the observed behaviours with linear, quadratic and general linear models with differing link functions tested for best fit. Models were compared using Log likelihood or the R^2 value and tested against model assumptions using the DHARMa package (Hartig 2020). The independent variables in each model included average wind speed (ms^{-1}), temperature ($^{\circ}C$) and insect species. Interactions between wind speed and temperature, bee species and wind speed and bee species and temperature were also included. Weights for 32 *B. terrestris/lucorum* foragers were collected. Models including weight as an independent variable were run, but weight never had a significant effect and, therefore, was excluded from the final models. Number of flower visits was log transformed and handling time was square root transformed. For inter-flower flight duration and proportion of flowers walked to general linear models were run with quasipoisson link functions. To test if temperature and average wind speed were correlated a Spearmans rank correlation was run. Due to multicollinearity between the predictor variables, they were standardised by subtracting the mean from each observed value (Iacobucci et al 2016). The data on honey bee foraging behaviour was used in a previous study (Chapter six, Hennessy et al *in submission*).

The number of *Bombus* foragers on a patch in relation to wind speed was investigated with a linear regression model in which total number of bees was the dependent variable with wind speed (ms^{-1}) and temperature ($^{\circ}C$) and the interaction between them were the predictor variables. A paired Wilcoxon-signed rank test was also run to determine if the number of foragers present on a patch was significantly different between patches when wind was or was not present within the same sample period.

In addition, the time a forager spent within a patch in relation to wind speed was investigated using linear regression, with wind speed as the only predictor variable and time spent within a patch log transformed.

7.4 Results

7.4.1 Foraging behaviour of Apis mellifera and Bombus terrestris/lucorum on Grosso lavender.

A total of 190 *A. mellifera* and 62 *B. terrestris/lucorum* foragers were studied foraging on lavender. Wind speeds ranged from 0-3.56ms^{-1} and temperatures between 19 -28°C. The average weight of a *B. terrestris/lucorum* forager was 0.29 ± 0.01mg, *N*=32. There was no correlation between wind speed and temperature (r=0.007, *P*=0.907)

7.4.1.1 Number of flower visits

Wind speed significantly reduced the number of flower visits for *A. mellifera* (linear regression, $t_{1,245}$=-8.78, *P*<0.001) but not for *B. terrestris/lucorum* (linear regression, $t_{1,245}$=-1.79, *P*=0.077, Fig 1a). *A. mellifera* visited 36% fewer flowers per minute at the highest wind speeds (>2.5m ms^{-1}) compared to the lowest (0-1 ms^{-1}), 14.3± 0.46, *N*= 35 vs 20.5 ± 0.94, *N*=59. The interaction between wind speed and bee species was significant (linear regression, $F_{1,245}$=9.14, *P*=0.003) as was the interaction between temperature and insect species (linear regression, $F_{1,245}$=36.01, *P*<0.001). Number of flower visits for *B. terrestris/lucorum* significantly increased with temperature (linear regression, $t_{1,245}$=6.05, *P*<0.001, Fig 1b), with a 38% increase from 22.81 ± 2.62 *N*=16 to 33.42 ± 1.86, *N=26* (19 vs 27°C). Temperature had no effect on *A. mellifera* flower visits (linear regression, $t_{1,245}$=-1.95, P=0.052). The interaction between wind speed and temperature was not significant (linear regression, $F_{1,245}$=0.02, P=0.891) for either species.

7.4.1.2 Flower Handling time

Wind speed significantly increased handling time for *A. mellifera* (linear regression, $t_{1,245}$=9.82, *P*<0.001) but not for *B. terrestris/lucorum* (linear regression, $t_{1,245}$=2.01, P=0.50, Fig 1c). However, the interaction between wind speed and insect species was also significant (linear regression, $F_{1,245}$=9.00, P<0.001). At the highest wind speeds (>2.5 ms^{-1}) *A. mellifera* handling time per flower averaged 3.82s ± 0.16s, *N*=35 compared to 2.70s± 0.08s, N=59 when there was no to little wind (0-1 ms^{1}), a 34% increase. The interaction between bee species and temperature was significant (linear regression, $F_{1,245}$=60.89, *P*<0.001), with *B.*

terrestris/lucorum handling time following a quadratic trend with temperature (quadratic regression, $t_{1,245}$=7.14, *P*<0.001, Fig 1d). The highest mean handling times at the lowest and highest temperature (19°C: 2.59 ± 0.27s, *N*= 16, 27°C:1.75 ± 0.21s, *N*=26) with the lowest mean handling time at mid-range temperatures (24°C: 1.25 ± 0.30s, *N*=13). *A. mellifera* handling time was unaffected by temperature (linear regression, $t_{1,245}$=-1.12, *P*=0.266). The interaction between temperature and wind speed was not significant ($F_{1,245}$=1.03, *P*=0.310).

7.4.1.3 Flight bout duration

Flight bout duration did not vary significantly with wind speed for either *A. mellifera* or *B. terrestris/lucorum* (linear regression, $F_{1,245}$=0.07, *P*=0.785, Fig 1e). Neither the interaction between wind speed and temperature nor wind speed and bee species were significant ($F_{1,245=}$0.36, *P*=0.550, $F_{1,245}$=0.35, *P*=0.557). However, the interaction between bee species and temperature was ($F_{1,245}$=10.96, *P*<0.001). Flight bout duration significantly increased with temperature for *A. mellifera* ($t_{1,245}$=3.78, P<0.001) but no effect was seen for *B. terrestris/lucorum* ($t_{1,245}$=-1.53, P=0.128, Fig 1f). Mean *A. mellifera* flight bout duration increased by 59% between the lowest and highest temperatures (19°C: 0.91 ± 0.12s, *N*=31, 28°C: 1.67 ± 0.25s, *N*=5).

7.4.1.4 Proportion of flowers walked to

The interaction between wind speed and insect species was significant (GLM, $F_{1,245}$=6.04, *P*=0.015), with wind speed alone significantly reduced the proportion of flowers walked to for *A. mellifera* ($t_{1,245}$= -2.64, *P*=0.009) but having no effect on *B. terrestris/lucorum* ($t_{1,245}$= 0.90, *P*=0.371). *A. mellifera* walked less between flowers compared to *B. terrestris/lucorum* in increasing wind speeds (Fig 1g). At the highest wind speeds, 2.5-3ms^{-1}, *A. mellifera* flew to 60% of flowers versus 83% for *B. terrestris/lucorum*. The interaction between insect species and temperature was also significant (GLM, $F_{1,245}$=5.63, *P*=0.015). However, temperature alone was not significant for either species (*A. mellifera:* $t_{1,245}$= -1.81, *P*=0.072. *B. terrestris/lucorum*: $t_{1,245}$= 1.57, *P*=0.012). *B. terrestris/lucorum* walked to more flowers with increasing temperature whereas *A. mellifera* walked to fewer (Fig 1h). Between the lowest and highest temperatures (19 and 28°C) *A. mellifera* walked between flowers 75% less compared to *B. terrestris/lucorum* foragers, who walked 15% more.

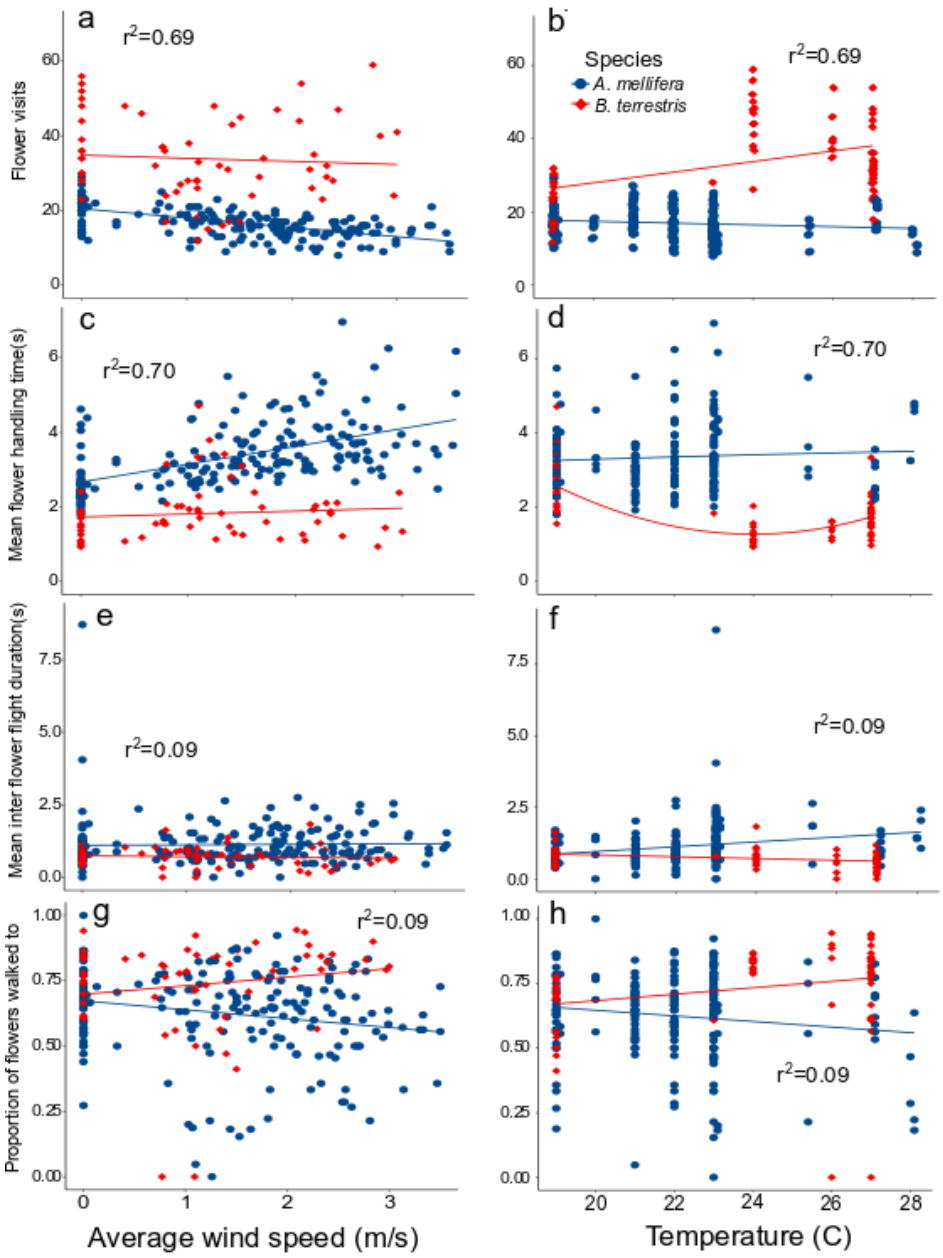


Fig 1: Foraging behaviours of *A. mellifera* and *B. terrestris/lucorum* workers foraging for a minute on either *Lavandula x intermedia 'Grosso'.* a-b Number of flower visits in relation to a: wind speed, b: temperature, c-d mean duration of flight bout in relation to c: wind speed and d: temperature, e-f mean duration of flight bout in relation to e: wind speed and f: temperature, g-h the proportion of flowers walked to in relation to g: wind speed and h: temperature. Linear regression lines are shown with r^2 values. *N*=190 for *A. mellifera* and *N*=62 for *B. terrestris/lucorum* for all behaviours

7.4.2 Number of Bombus foragers on Lavandula x intermedia 'Grosso' with and without wind

Wind speed ranged from 0-3.3ms^{-1} and temperature ranged between 18-27°C with a total of 158 individual bees included in the analysis. Due to the high number of zeros for individual species all species were pooled, with a total of five *Bombus* species recorded.

Increasing wind speed significantly reduced the number of foragers within a patch (GLM, $F_{1,156}$=34.84, P<0.001, Fig 2a), while increasing temperature significantly increased the number of foragers (GLM, $F_{1,155}$=22.88, P<0.001, Fig 2b). The average number of foragers in a patch with little or no wind (0-1ms^{-1}) was 4.38 ± 0.25, N=107, 59% more compared to higher wind speeds (>2ms^{-1}), where the average was 2.38 ± 0.51, N=26. The interaction between temperature and wind speed did not significantly influence the number of foragers present in a patch (GLM, $F_{1,154}$=0.26, P=0.613).

There was a significant difference in the number of *Bombus* spp. foragers in a patch that was experiencing wind vs the adjacent patch in the same sampling period where no artificial wind was present (Paired Wilcoxon-Signed rank test, V=945, P<0.001). The average number of *Bombus* foragers in a patch with no wind was 4.43 ± 0.26, N= 99, compared to 2.46 ± 0.34, N= 59, in a patch experiencing wind (Fig 2c).

7.4.3 Time spent in patch

Data on time spent in patch were collected from 70 individual bees in wind speeds ranging from 0-3ms^{-1}. The time spent foraging in a patch significantly decreased with wind speed (linear model, $F_{1,68}$=7.36, P=0.008, Fig 2d). When no wind was present the average time spent foraging in a patch was 49.4 ±8.84(s), N=25. This decreased by 99% to 16.77± 5.09(s), N=8 at the highest wind speeds (2.5-3ms^{-1}).

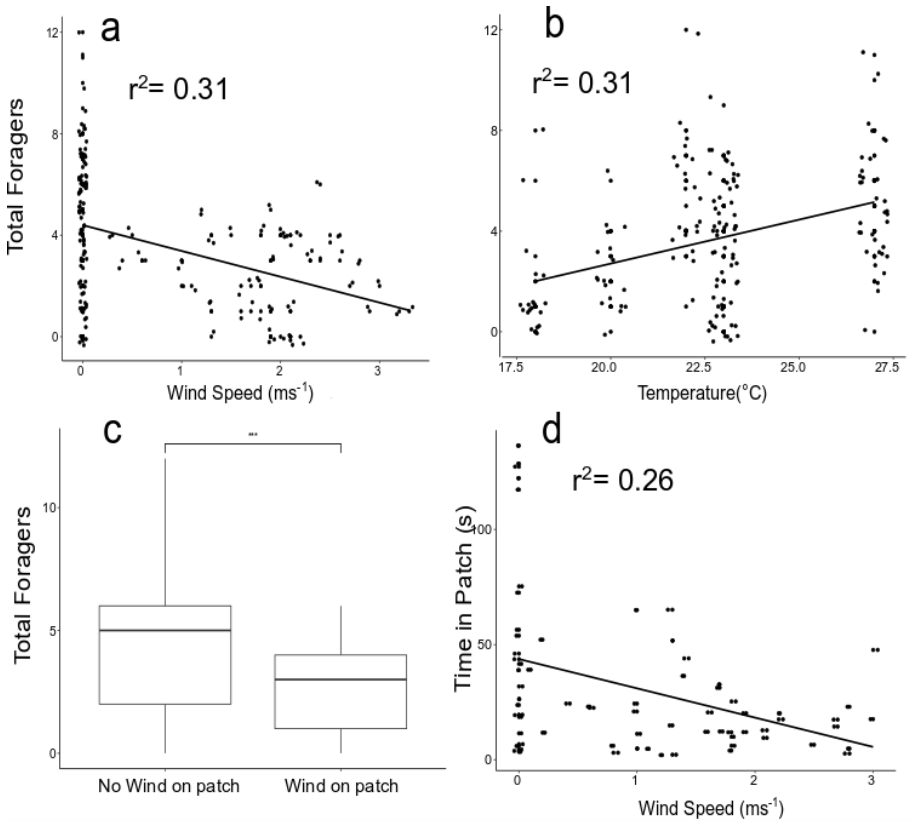


Fig 2: The number of foragers in a patch of *Lavandula x intermedia* 'Grosso' in relation to a: wind speed (ms^{-1}) , b: temperature (°C) and c: the presence or absence of wind on the patch and d: the time (s) foragers spent within a patch in relation to wind speed. Lines shown are regression lines with the r^2 value.

7.5 Discussion

Our results indicate that both wind speed and temperature affect bee foraging, but that the effects differ between honeybees and bumblebees. For example, *A. mellifera* flower visiting rate was significantly reduced with increasing windspeed, with a reduction of 36% from the lowest ($0ms^{-1}$) to highest wind speeds ($>2.5ms^{-1}$). However, in *B. terrestris/lucorum* flower visiting rate was unaffected by wind speed.

The reduction in flower visiting rate with increasing wind speed in *A. mellifera* matches previous research. Hennessy et al (2020) found a 37% decrease when wind speed increased from 0 to $2.75ms^{-1}$ when *A. mellifera* were foraging on stationary artificial flowers. In the current study the reduction in *A. mellifera* was due to an increase in flower handling time. Average handling time for *A. mellifera* increased from 2.70 to 3.82 seconds per flower

between low (0-1ms^{-1}) and high (>2.5ms^{-1}) wind speeds. In Hennessy et al (2020), the reduction in flower visits was due to foragers taking longer to take off from a flower ("increased hesitancy"). Although take off time was not directly examined in this current study, handling time incorporated all activities whilst a bee was on a flower, including take-off, probing and walking between flowers. We can hypothesise that the increased handling time with wind may be due to *A. mellifera* taking longer to take-off from a flower.

By contrast, for *B. terrestris/lucorum* both the flower visiting rate and handling time per flower were unaffected by wind speed. Although we cannot explain the exact reasons why the effects of wind are different between honeybees and bumblebees, we hypothesise that body size may be a factor. In our study, *B. terrestris/lucorum* average weight was 290mg compared to c. 129mg for the average *A. mellifera* forager (see Otis 1982). Air resistance is more of an issue for lighter animals (Hunter 2007) as they have a greater surface area to volume ratio. Being larger may make behaviours, such as take-off, easier in higher winds.

Although being larger may help with taking off in higher wind speeds, it could make navigating moving obstacles more difficult. This current study found that whilst foraging on lavender in increasing wind, *A. mellifera* flew to proportionally more flowers compared to *B. terrestris/lucorum*. Previous research has shown that larger *B. terrestris* take longer to complete foraging trips in cluttered environments with no wind due to difficulties in manoeuvrability and avoiding collisions (Crall et al 2015). *A. mellifera* have been found to adopt a different strategy. When foraging in wind and navigating through moving obstacles they tend to increase their flight speed, to reduce the amount of time spent in these unfavourable conditions (Burnett et al 2020). These different strategies may explain why *A. mellifera* tended to fly between flowers more in wind compared to *B. terrestris/lucorum* when foraging on lavender.

However, and perhaps surprisingly, the inter-flower flight time was unaffected by wind speed for both bee groups. The methods by which bees stabilise themselves in flight in response to wind is well studied for both *Bombus* spp. and *A. mellifera*. They use a combination of passive and active changes to body positioning in response to the forces combined with wing beat frequency changes to stabilise body positioning (Ravi et al 2016 and Crall et al 2017). These adjustments presumably allow bees to fly between flowers efficiently, even in windy conditions. Wind also indirectly affects foraging bee movements by causing flowers to 'sway'. Lavender flowers sway more at higher wind speeds (Hennessy et al in press).

However, previous work on both artificial and real flowers has found that flower movement can improve landing accuracy (Kapustjansky et al 2010) and flowers which move in the wind are also generally more attractive than those which are stationary (Warren and James 2008). This may be because movement of objects against a stationary background aids visual detection by bees (Lehrer and Srinivasan 1992). Perhaps some increased flower movement in wind makes landing easier, negating any in-flight challenges, such as being blown off course, which would otherwise increase flight time between flowers.

Although *B. terrestris/lucorum* foraging rate on lavender was unaffected by wind speed, increasing temperature resulted in an increased flower visiting rate. This was due to a decrease in handling time, except at the highest temperatures (27°C) where handling time began to increase again. This could be due to a variety of factors. Bumblebees generally take off faster from flowers in warmer temperatures (Heinrich 1975). However, this increase in take-off speed may be negated by difficulties in extracting nectar at higher temperatures as it becomes more viscous and harder to extract (Corbet 1979, Nardone et al 2013).

The number of *Bombus* foragers on a lavender patch decreased with increasing wind speed (Fig 2a). The wind was localised on a single patch of lavender, with neighbouring patches not experiencing wind. There were significantly more *Bombus* foragers on a patch without wind versus with wind at the same time, perhaps due to bees choosing to forage on the wind free patches. Bumblebees make foraging decisions based on profitability (Cnaani et al 2006) and although the number of flower visits was unaffected by wind, flying in windy conditions requires more energy (Wolf et al 1999). Therefore, it is likely when a choice is available individuals will travel to a patch where it is less windy. Not only were there fewer foragers in higher winds, but bees also spent less time foraging. If by continuing to forage in a windy patch the energy lost is higher than the energy gained, then it would make sense for foragers to leave and try to find a less windy patch, such as one in a sheltered position. When foraging *A. mellifera* and *Bombus* species expend approximately half the energy gained through nectar collection (Balfour et al 2015, Balfour et al 2021). In other words, small energy gains or reductions in costs may well be worth having given such a tight energetic tightrope, such as by locating a more sheltered patch.

The significant reduction in flower visits by *A. mellifera* seen in this study may help explain why their foraging activity is reduced in windy conditions (Pinzauti 1986, Javorek et al 2002, Tuell and Isaac 2010). Honeybees make an estimated 250 to 1446 flower visits per foraging

trip depending on the plant species being foraged (Goodwin et al 2011 and Ribbands et al 1949), meaning a 36% reduction in visits per minute could have a significant impact in energetic returns, making not foraging potentially the more profitable decision. This is especially the case if other insect species foraging on the same plant are less affected by wind, increasing the potential for competition and reducing energy returns even further (Balfour et al 2015). Studies examining forager activity in natural wind conditions have shown that *A. mellifera* are particularly sensitive to increasing wind speed, with forager activity and flower visit rate more severely affected compared to other wild bee species (Vicens and Bosch 2000, Brittain et al 2012). Although this current study is limited in that we have only examined two bee groups foraging on lavender, the results indicate that there are differences the responses of different bee species to wind. Often wind speeds will be above the maximum seen in this study ($3.56ms^{-1}$) and if certain bee species foraging efficiency is reduced, it may be possible for other pollinators to fill the pollination deficit if they are less affected by increasing wind. For example, in apple orchards with high pollinator diversity high winds ($>2.5ms^{-1}$) did not cause a reduction in overall flower visits, whereas in orchards with low diversity number of visits significantly declined (Brittain et al 2012).

Our study has identified that *B. terrestris/lucorum* are less affected by wind than *A. mellifera* when foraging on lavender. The reduction in flower visitation rate by *A. mellifera* was due to an increase in flower handling time, with flight between flowers unaffected, highlighting how bee species' responses to wind are complex. Factors such as differences in body size likely play a role in a species' ability to forage in wind. However, other non-physical aspects, such as competition between species, may also be contributing (Vicens and Bosch 2000, Brittain et al 2012, Balfour 2015). Global wind speeds are currently increasing (Zeng et al 2019). By understanding species specific responses to increasing wind speeds measures, such as providing biodiverse habitats (Brittain et al 2012) can potentially be put in place to help buffer the reduction of activity seen with bee species like honeybees. More work on a range of pollinator species is required to fully understand the mechanisms that cause these differences in behaviour, and to also determine if these responses are universal when foraging on different plant species.

Chapter Eight: Wind beneath my wings: Increasing wind speed causes increased hesitancy to take-off in foraging honeybees and bumblebees

8.1 Abstract

Wind is an environmental factor which can affect flying insects. Previous research showed that increasing wind caused an increase in take-off hesitancy for honey bees foraging on artificial flowers. This study determined whether take off hesitancy occurs when bees forage on natural plants. Honey bees and bumble bees foraging on seven plant species were video recorded across a range of wind speeds. Hesitancy to take-off from a flower was quantified, this was the time between when a bee stopped probing for nectar to when it took flight. Hesitancy increased with increasing wind speed by an average of 50% for both bee types. Flower movement in increasing wind speed was also quantified by video recording flowers of the study species in a range of wind speeds. Bumble bee hesitancy increased more with increasing flower movement when compared to honey bees. Flower movement alone did not significantly influence hesitancy times for either honey bees or bumblebees. This is the first study to show that increasing wind speeds can cause an increase in take off hesitancy in nature and affect the foraging performance of two important pollinators, honey bees and bumble bees. Foraging bees on average can visit up to 20 flowers per minute, meaning any increase in handling time, even if small, could potentially result in a significant reduction in flower visit rate.

8.2 Introduction

When foraging, animals encounter an array of factors that can influence behaviour and efficiency. These factors can be biotic, such as competition (Grand and Dill 1999, Balfour et al 2015, Wignall et al 2020) and predation risk (Lima et al 1985, Jones and Dornhaus 2011, Bonnot et al 2012) and abiotic such as temperature (Fraser et al 1993, Edwards et al 2015, Funghi et al 2019), rainfall (Radford et al 2001, Chard et al 2017, Farji-Brener 2018) and wind (Alma et al 2016, Lane et al 2019, Hennessy et al 2020).

Wind is a particularly important factor for flying animals. It varies more rapidly than other abiotic conditions (often within seconds) and has considerable energy (Stull 1988). Wind can also vary across a range of spatio-temporal scales and is known to affect the efficiency, behaviour and energetics of flying animals (Tucker and Schmidt-Koenig 1971, Alerstam 1979, Wolf 1999, Chapman et al 2010). Take-offs and landings are often influenced by wind (McMillan 1940, Cone 1964, Chang et al 2016). For example, when landing at the nest in strong wind conditions, cliff landing auks fail 60% of attempts (Shepard et al 2019), and strong winds generally decrease birds propensity to take-off for migration events (Liechti 2006). Take off is also an energetically expensive part of flight (Weimerskirch et al 2000), with power equivalent to many times the body weight of the animal required (Alexander 2003). Although wind can make take-off more difficult, it can also reduce the energetic costs associated with it (Clay et al 2020), with increasing wind speed found to cause a reduction in take-off duration for some bird species (Kogure et al 2016).

Take-off in relation to wind speed for flying insects is less studied than for birds. When foraging, bees, can visit 10-20 flowers per minute (Couvillon et al 2015), with many take-offs required to travel between individual flowers and inflorescences. Although increasing wind can reduce the take-off energy cost in birds, insects operate on a much smaller scale. Smaller animals have lower maximal lift and higher drag, reducing the aerodynamic efficiency of small wings (Roderick et al 2017). Air resistance is also more of an issue for lighter animals (Hunter 2007) as they have a greater surface area to volume ratio. These factors mean that increasing wind may not only make sustaining flight harder for flying insects (Wolf 1999) but may also influence take-off.

Previous work studying wind and insect flight has mainly focused on wing dynamics during flight (Willmott and Ellington 1997, Ravi et al 2016, Burnett 2020), how wind decreases foraging activity (Pinzauti 1986, Tuell and Isaacs 2010) and how landing can be impacted

(Chang 2016). The few studies that have examined take-off behaviour have highlighted how take-off activity decreases in increasing wind speeds (Walters and Dixon 1984), with individuals often waiting for a lull in the wind to initiate flight (Messing et al 1997). Increasing wind speed has also been found to reduce foraging performance in honey bees both foraging on natural and artificial flowers (Hennessy et al in press, Hennessy et al 2020), due to an increase in handling time. Hennessy et al (2020) found that this increase in overall flower handling time was due to greater take off hesitancy, that is taking longer to take-off from a flower in increasing wind. However, this previous study used stationary artificial flowers. To our knowledge, there has been no work examining if take off hesitancy also occurs on natural flowers.

In Hennessy et al (2020) the direct effects of wind on the bee and the indirect effects of wind on flower movement were studied separately. Flowers can sway in the wind, with various factors, such as stalk length, influencing movement (Warren and James 2008). Flower surfaces also have a variety of cell structures, some that even improve pollinator grip (Whitney and Federle 2013). Studies have shown that bumble bees will choose plant species that have easier to grip flowers when foraging on moving plants (Whitney et al 2009), highlighting that flower movement does influence foraging ability. However, how it influences take-off is not known.

The aim of this study is to determine whether honey bees (*Apis mellifera*) and bumble bees (*Bombus* sp.), which are both important pollinators, have increased take-off hesitancy with increasing wind speed when foraging on a range of plant species, and to make comparisons between the 2 bee types and the seven flower species studied. In addition, flower movement was also quantified to determine if this also influences take-off hesitancy.

8.3 Methods

8.3.1 Chosen plant species

We studied free flying *Apis mellifera* and *Bombus* species foraging on a range of plant species growing wild under natural wind conditions. Plant species were chosen to ensure that a variety of flower morphologies were studied. Seven different plant species were studied from six families. *Rubus fruticosus* (bramble), *Cirisium arvense* (creeping thistle), *Centaurea scabiosa* (greater knapweed), *Echium vulgare* (vipers bugloss), *Jacobaea vulgaris* (ragwort), *Trifoliuim repens* (white clover) and *Melilotus officinalis* (yellow sweet clover).

Study patches were chosen to have exposure to wind along with patch size to be large enough to have many foraging insects to avoid repeat recordings on the same insect, or alternatively, there were multiple smaller patches within 50m. Each plant species was studied at one location. Data collection occurred on both windy and still days without rain and in temperatures above 15°C. Data collection occurred on 25 days between 16 June and 29 August 29 2020.

8.3.2 Video recording bees

A multi directional anemometer (Skywatch Eole) was placed on a tripod within the study patch at average flower height for the species being studied. A Sony camera (HDR-CX115) was attached to a tripod to video record anemometer wind speed. When a suitable foraging bee was seen it was recorded by a second video camera (Sony HDR-CX115) held by the observer at 24 frames per second. The behaviour being examined was the hesitancy to take-off from a flower. This was defined as the time between when the forager finished either probing for nectar or collecting pollen on a flower to taking flight

Inflorescences with multiple flowers close together, on which bees would walk between flowers, were treated as a single flower. This was the case for *J. vulgaris*, *C. arvense* and *C. scabiosa*. However, when flowers were further apart on the same inflorescence and bees flew between them, each flower was treated separately. This was the case for all other plant species studied. If a bee walked between flowers it would be video recorded until it took flight.

When a bee was sighted foraging within the study patch, the camera recording the anemometer would be turned on and the wind speed from when a bee started foraging on a flower to when she took off would be recorded. This allowed the video to be viewed to note the wind speed on take-off

Only one take-off per bee was recorded. It was not possible to capture and paint mark bees to totally exclude the possibility of studying the same bee twice. However, to reduce the chance every 10 minutes the focal plants would be changed. If the same bee was videoed twice, it would have been a separate visit to a flower under a different wind condition.

The temperature (±1°) was recorded after each bee recording using a thermometer (KTJ) placed at average flower height.

8.3.3 Video playback

Videos were imported into the software MPEG streamclip. This programme allowed the recording to be played frame by frame and the hesitancy to take off from a flower to be accurately quantified. This was calculated from when a bee stopped probing for nectar, which was defined as the first frame when the bee visibly began to pull the tongue away from the nectary, or when she stopped collecting pollen, which was defined as when she was no longer seen to be rubbing the body and or scopa, when the mouthparts were no longer to be seen moving, or when the antennae were no longer bent and actively contacting the flower. Behaviours which were not included as either probing for nectar or collecting pollen were walking around the flower with no signs of rubbing the body for pollen collection with the antennae raised, and cleaning of the tongue. Take-off was recorded from the first frame where no body parts (e.g. feet) were touching the flower and the bee was therefore fully in flight. Whether the bee was foraging for pollen or nectar was also recorded.

The bee species recorded were *Apis mellifera* (honey bee), *Bombus terrestris/lucorum,* which were combined due to difficulties in identification in the field and *Bombus lapidarius*. These were the only bumble bees sufficiently abundant for the study. Some data were collected on *Bombus pascorum*. However, because the sample size was very low this species was removed from the study.

8.3.4 Quantifying flower movement

To quantify flower movement across wind speeds, flowers from all study species were videoed with a Sony camcorder (HDR-CX115) on a tripod placed at average flower height 2m away from a reference point within a study patch, which was marked with a bamboo pole. An anemometer was placed within the patch at flower height to measure wind speed. To ensure the same flower, or inflorescence in the three species where an inflorescence was treated as a flower, was not studied twice, multiple patches within the same location were recorded. Flowers were selected to ensure flowers from a range of heights were analysed. Videos were taken in natural wind speeds from 0-6.5ms^{-1} and recorded at 24 frames per second. Videos were imported into the software imageJ and individual flowers were chosen at random from the reference point and their location tracked in each frame for 100 frames (4 seconds). For each frame the wind speed was also recorded. The average wind speed and flower movement (cms^{-1}) was calculated for each flower, with 30 flowers per plant species.

Due to the footage not being recorded in 3-dimensions. movement was calculated on the horizontal (x) and vertical (y) plane, with longitudinal (z) movement ignored. This method has been used in previous work assessing leaf movement (Kothari and Burnett 2017).

8.3.5 Statistical analysis

Statistical tests were performed in R 3.3.3 (R Development Core Team 2009). A linear regression model was run with hesitancy at take-off log transformed as the dependent variable. A generalised linear mixed effects model with location as a random effect was tested. However, location did not explain significantly more of the variance and so was dropped from the model. A mixed effects model was also tested with plant species as a random error. However, when comparing model fit using the DHARMa (Hartig 2019) package the linear model was the best fit and therefore used for the analysis. To determine if flower movement influenced hesitancy duration, average flower movement for each plant species was calculated from three wind speed categories, when wind speed ranged from 0-1ms^{-1}, 1.1-3 ms^{-1}, and above 3ms^{-1}. These values were then matched with bees which had experienced wind speeds at take off within the same categories. Plant species, wind speed at take-off, bee group, temperature and flower movement were predictor variables. The interactions between plant species and wind speed, plant species and temperature, plant species and bee group, average movement and bee group and average movement and temperature were also included. The interaction between temperature and wind speed was found to be non-significant and so was removed. Nectar or pollen collection alone and its interaction with wind speed were included in the models but also found to never be significant so were removed. The interaction between bee group and wind speed was not significant so was removed form the final model. To account for multicollinearity in the model all predictor variables were centred by subtracting the mean from each observed value (Iacobucci et al 2016). All values with a variance inflation factor (VIF) value of <3 were retained in the full model (Zuur et al 2010). There was no significant difference in hesitancy to take-off between *B. terrestris/lucorum* and *B. lapidarus*, nor was the interaction between wind speed and bee species significant, therefore both species were grouped for analysis. The final model was:

Log(Hesitancy duration)~ Average flower movement*Wind speed + Average flower movement*Temperature + Average flower movement*Bee group+ Plant Species*Bee group + Wind speed *Plant Species

To determine if flower movement was influenced by wind speed a linear regression was run with average flower movement log transformed as the dependent variable. Average wind speed and plant species were the predictor variables. The interaction between average wind speed and plant species was also included.

Table 1: Plant species included in study and where they were located in Sussex, England. For plant species where multiple spaced apart patches were used plants were within 50m

Plant species	Location of data collection	Grid Reference
Rubus fruticosus	Sheepcote Valley	TQ 34022 04961
Cirisium arvense	Stanmer Park	TQ 34157 08771
Centaurea scabiosa	Brighton Racecourse	TQ 33570 05756
Echium vulgare	University of Sussex campus	TQ 34281 09709
Jacobaea vulgaris	Brighton Racecourse	TQ 33378 05723
Trifoliuim repens	Falmer Village	TQ 38876 21714
Melilotus officinalis	Sheepcote Valley	TQ 34075 05399

8.4 Results

Data were collected from a total of 827 *A. mellifera* and 898 *B. terrestris/lucorum* and *B. lapidarius* foragers across the 7 plant species. Wind speed at take-off ranged from 0-6.5ms^{-1} and temperatures from 16.5-38C.

Hesitancy to take off significantly increased with increasing wind speed for both bee groups (LM, $F_{1,1696}$=71.61, P<0.001, Fig 1a), with an average increase of 50%, 0.19s, for both honey bees and bumble bees between the lowest (0-1ms^{-1}) and highest (3-5ms^{-1}) wind speeds. Hesitancy to take off was significantly different between the two bee groups independently of wind speed, with honey bees on average more hesitant (LM, $F_{1,1696}$=64.39, P<0.001). Across wind speeds, average hesitancy for honey bees was 0.40 ± SE 0.01s compared to 0.32 ± SE 0.01s for bumble bees.

The interaction between plant species and wind speed was not significant ($F_{6,1696}$=1.24, P=0.284, Appendix E3-4). For *A. mellifera* average hesitancy across all plant species at low wind speeds (0-1ms^{-1}) was 0.36 ± SE 0.02s, N=355. This increased by 44% at the highest

wind speeds (3-5ms^{-1}) to 0.52 ± SE 0.05s, *N=63.* For *Bombus* species hesitancy increased by 56% between the same wind speed categories (0.27 ± SE 0.02s, *N*=372 vs 0.42 ± SE 0.04s, *N*=92).

The interaction between bee group and plant species was significant (LM, $F_{6,1696}$=5.87, P<0.001, Fig 1b). *Rubus fruticosus* (bramble) was the plant species where average hesitancy per flower was highest for *A. mellifera*, $\bar{X}$ =0.51 ± SE 0.05s, with *Bombus* average hesitancy being 49% lower. For *Bombus,* average hesitancy was highest on knapweed, $\bar{X}$ =0.39 ± SE 0.02s with *A. mellifera* hesitancy 14% higher, $\bar{X}$ =0.45 ± SE 0.03s. When foraging on the same plant species hesitancy to take-off significantly differed between bee groups on bramble (*t*=6.91, P<0.001, *A. mellifera* $\bar{X}$= 0.51 ± SE 0.05s vs *Bombus* $\bar{X}$= 0.31 ± SE 0.02s) and vipers bugloss (*t*=8.59, *P*<0.001, *A. mellifera* $\bar{X}$= 0.47 ± SE 0.02s vs *Bombus* $\bar{X}$= 0.30 ± SE 0.01s). For all other plant species comparisons see Appendix E1.

Hesitancy was significantly affected by temperature (LM, $F_{1,1696}$=8.27, *P*=0.004), with hesitancy decreasing with increasing temperature for both bee groups.

The interactions between bee group and wind speed (LM, $F_{1,1696}$=0.08, P=0.775) and temperature and plant species (LM, $F_{6,169\,6}$= 1.39, *P*=0.215) did significantly influence hesitancy to take off.

The interaction between flower movement and bee group was significant (LM, $F_{1,1696}$=10.77, *P*=0.001 Fig 1c). Flower movement alone was not significant for either *Bombus* species (t=-0.219, *P*=0.827) nor *A. mellifera* (t=0.416, *P*=0.677, Appendix E2). *Bombus* hesitancy increased more with flower movement compared with *A. mellifera* (Fig 1c). Flower movement ranged from 0.15cms^{-1}, with bramble in wind speeds of <1ms^{-1}, to 32cms^{-1} with *Jacob vulgaris* (ragwort) in wind speeds >3ms^{-1}. *Bombus* average hesitancy increased from $\bar{X}$= 0.27 ±0.02s, *N*=82 at an average flower movement of 0.15cms^{-1}, to $\bar{X}$ =0.34 ±0.03s, *N=45* at an average flower movement of 16.34cms^{-1}. For *A. mellifera,* at the same average flower movement hesitancy decreased from $\bar{X}$ =0.48 ±0.05s, *N*=72 to $\bar{X}$ =0.44 ±0.04s *N*=50.

8.4.1 Flower movement in increasing wind

Increasing wind speed significantly increased flower movement ($F_{1,224}$=141.9, P<0.001, Fig 2). The interaction between plant species and wind speed was significant ($F_{7,244}$=3.50, P=0.001) as was plant species alone ($F_{7,224}$=67.21 P<0.001). The plant species with the highest increase in movement speed was ragwort. At the lowest wind speeds (0-1ms^{-2}) the

average flower movement was 7.91±028 cms^{-1}, which increased by over 300% to 32.2±1.57 cms^{-1} at wind speeds between 3-5ms^{-1}, and by over 600% to 56.78 ±2.92cms^{-1} at the highest wind speeds (over 5ms^{-1}). *Centaurea scabiosa* (knapweed) was the only plant species where flower movement did not significantly increase with increasing wind speed (t=0.41, P=0.684), with a 5% increase in flower movement between the lowest (0-1ms^{-1}) and highest wind speeds (3-5ms^{-1}).

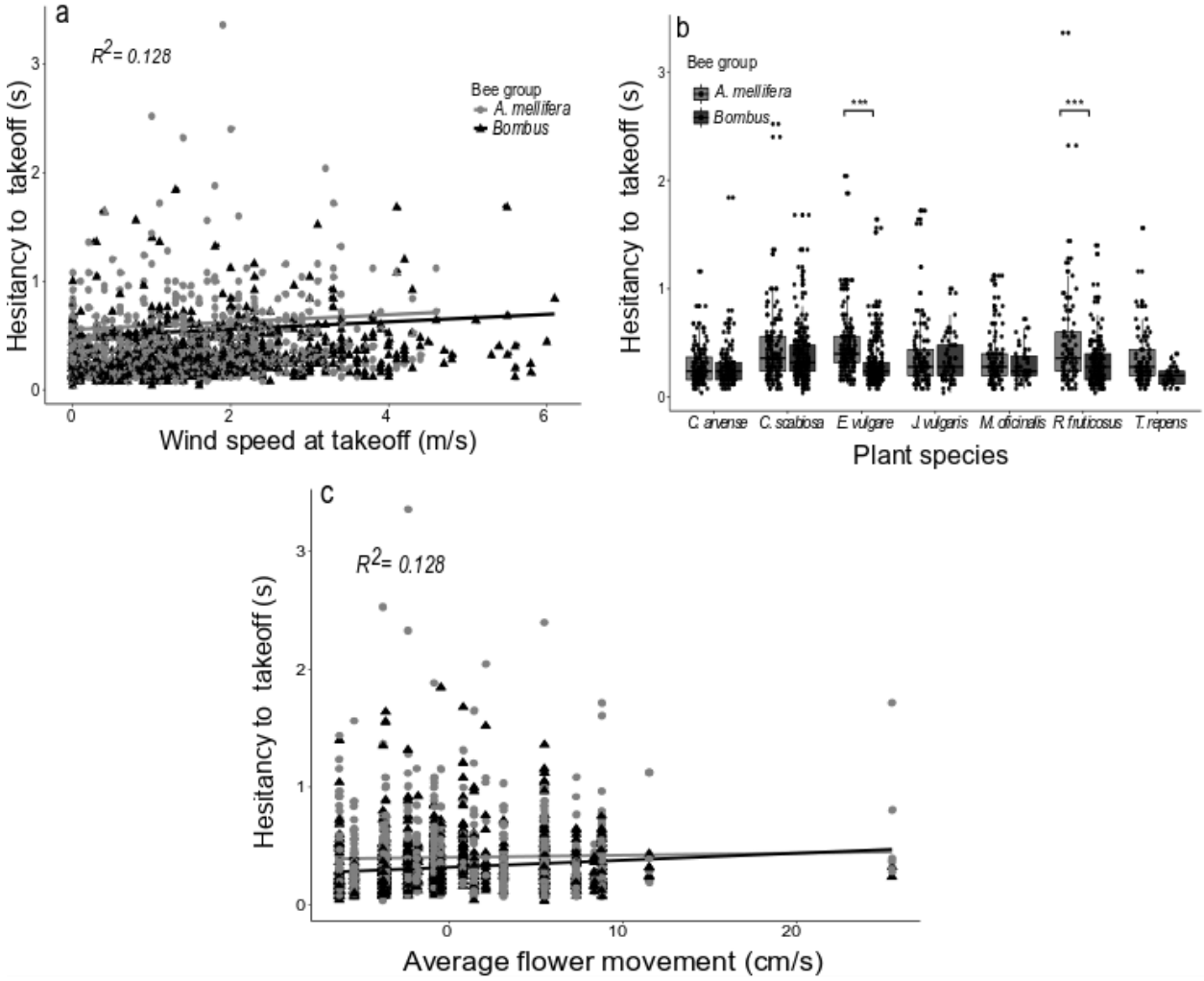


Fig 1: A: Hesitancy (s) to take off from a flower in relation to increasing wind speed at take-off (ms^{-1}) when foraging on all plant species, with linear regression lines and the corresponding R^2 value shown. *N*=827 for *A. mellifera* and *N*=898 for *Bombus* species. B: Hesitancy to take off for both *A. mellfiera* and *Bombus* species in relation to the plant species being foraged. The three asterisks signify where a significant difference (P<0.001) between bee groups was found when both were foraging on the same species. For a full table of significances see Appendix E1 C: Hesitancy to take off in relation to flower movement when plant species flower movements were calculated from three wind categories (<1$ms^{-1,}$ 1.1-3ms^2, 3.1-5ms^{-1}).

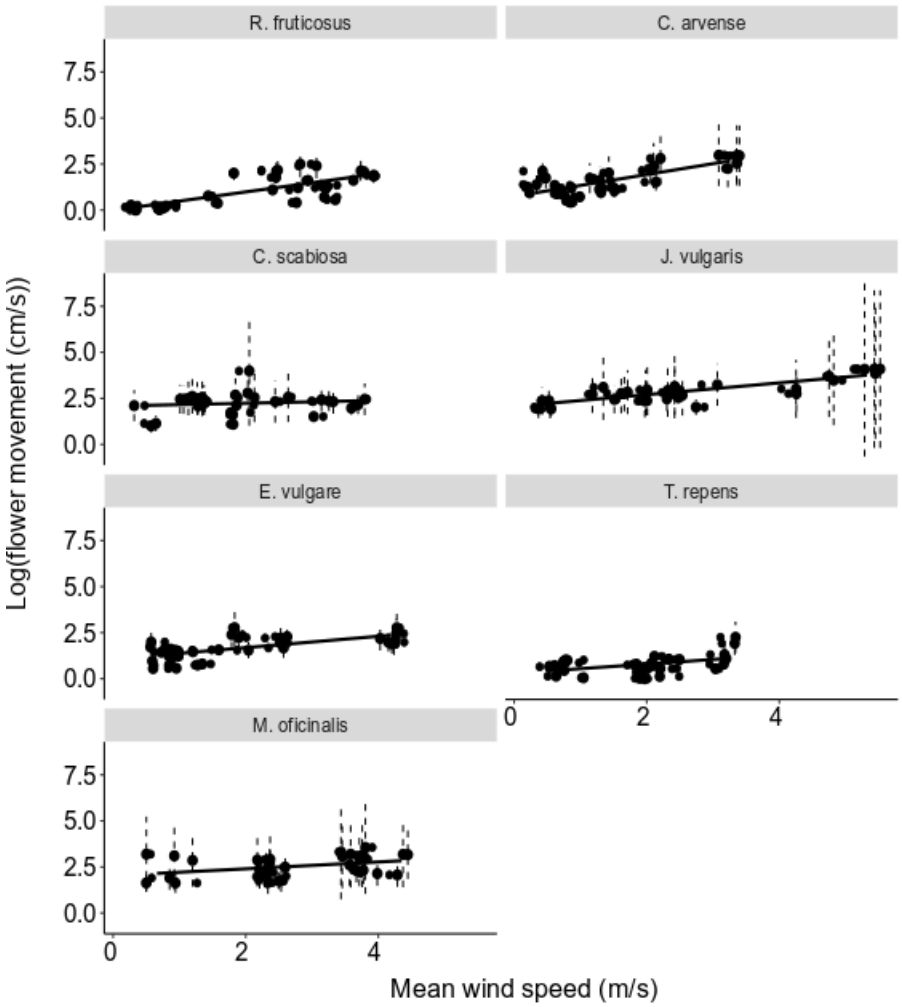


Fig 2: Logged average flower movement (cms^{-1}) against mean wind speed (ms^{-1}) for all studied plant species. Standard error bars are shown and the R^2 value was 0.72. Average flower movement was taken from 30 flowers for each plant species from 100 frames of footage. Dashed lines indicate standard error bars.

8.5 Discussion

Our results show that hesitancy to take-off increases with increasing wind speed for bumble bees, *Bombus* species, and honey bees, *A. mellifera*. Averaged across plant species hesitancy per flower increased by 44% for honey bees and 55% for bumble bees from low ($<1ms^{-1}$) to high ($3\text{-}5ms^{-1}$) wind speeds. The effect of wind speed did not vary between the plant species being foraged. However, hesitancy to take-off independently of wind speed did.

Increased hesitancy to take-off in increasing wind speeds occurred in a previous study on honey bees. However, this used artificial flowers with wind generated by fans (Hennessy et al 2020). The current study is the first to show it occurs when bees are foraging on real flowers in natural wind. It is unclear why hesitancy increased with wind speed. However, when foraging, bees must make decisions on which flower to visit next, with variables such as nectar concentration (Hodges 1981, Williams 1997), predation risk (Dukas 2001, Jones and Dornhaus 2011) and local competition (Baude et al 2011, Balfour et al 2015, Wignall et al 2020) all influencing flower choice. Research has shown that honey bees can also detect and use wind direction as a cue for foraging decisions (Ravi et al 2016). Bumble bees have also been found to complete more downwind flights in windier conditions (Comba 1999), showing that they can incorporate wind into their foraging choices. When making these decisions pollinators must trade-off speed and accuracy (Chittka et al 2009). More complex foraging decisions, such as choosing between two similar coloured flowers, often result in longer decision times (Chittka and Spaethe 2007). By waiting to take-off from a flower, perhaps more information on wind direction and speed can be obtained, potentially minimising the risk of being blown in the wrong direction. Future work could determine if there is a relationship between flight direction and wind direction in windy conditions.

Temperature significantly decreased hesitancy duration for both bumble bees and honey bees. When in flight, both groups are able to maintain a higher body temperature compared to the ambient temperature through their flight metabolism (Heinrich 1974). When not in flight, such as when foraging from a flower, a bee's body temperature will begin to reduce. In order to initiate take-off the thorax must be a certain temperature, which can be maintained when on a flower by shivering (Waddington 1990). When foraging in warmer temperatures, the time required to reach this thoracic take-off temperature is less, decreasing hesitancy duration (Heinrich 1974 and Waddington 1990).

Although the effects of wind speed did not differ between plant species, hesitancy to take-off independent of wind speed did (Fig 1b, Appendix F1). In other words, bees took longer to take off from some flowers more than others. Hesitancy to take-off differed between bee groups, with honey bees on average taking longer to take-off than bumble bees. However, the only two plant species where hesitancy to take-off was significantly different between the bee groups were *E. vulgare* and *R. fruticosus*, with bumble bees taking off faster than honey bees on both. Faster handling times by bumble bees has been seen in other plant systems (Free 1968; Heinrich 1979; Willmer et al 1994; Balfour 2013). For example, Balfour et al (2013) found that bumble bees were 3 times faster on lavender (*Lavandula* sp.) compared to honey bees, this was in part due to their longer tongues allowing them to not have to place their heads as deep into the corolla tube, resulting in faster handling times. This difference in tongue length may explain why bumble bees had significantly lower hesitancy times on *E. vulgare* compared to honey bees in our study, as *E. vulgare* has a long corolla tube similar to lavender (Corbet 1978). The differences in hesitancy seen between the other plant species in our study are harder to explain. Many variables not examined have the potential to influence handling, and potentially hesitancy duration. For example, nectar concentration (Harder 1986, Waddington 1990), flower orientation and complexity (Laverty 1994, Giovanetti and Aronne 2013, Wang et al 2014) and the shape of cells on the petal surface which provide grip (Whitney et al 2009, Alcorn et al 2012). Future work could examine floral traits and how these may influence take off hesitancy and why it varies between bumble bees and honey bees.

We did examine flower movement in increasing wind and found that it significantly increased in relation to wind speed and varied across plant species. Flower movement has previously been found to be an attractive trait for pollinators, with moderately 'wavy' flowers attracting more pollinators than those which were stationary (Wolf & Zerrahn-Wolf 1937, Warren and James 2008). Hesitancy duration in relation to wind speed, perhaps surprisingly, did not differ between the bee groups. However, hesitancy in response to flower movement did. Bumble bee hesitancy duration increased more with flower movement when compared with honey bees (Fig 1c). The reason why there is a difference in response between the two bee groups is unclear. Plant species are known to have different flower surface structures, some providing better grip than others (Papiorek et al 2014), with foragers choosing species with better grip when flowers are moving (Alcorn et al 2012). Honey bees have highly complex tarsi, allowing a strong grip even on smooth surfaces (Brauer et al 2017). However,

bumble bees have small tarsal pads in comparison to their body size, making their grip less secure (Whitney et al 2009). This possible relationship between increased grip and flower structure may result in honey bees being less affected by flower movement compared to bumble bees. The effects of flower movement seen in this study must be taken with some caution. Increasing flower movement is correlated with increasing wind speed, meaning bees will rarely experience low wind speeds on fast moving flowers, making it hard to disentangle the effects of wind or the effects of movement. In Hennessy et al (2020), artificial flowers were moved in the absence of wind, with the hesitancy duration of foraging honey bees unaffected. This agrees with results seen in this current study. However, to fully disentangle the effects of wind speed and flower movement similar manipulation studies on natural plants is required.

Wind is an environmental variable capable of influencing many aspects of the bee foraging process. Previous work has highlighted its impacts on flight mechanics (Ravi et al 2013, Crall et al 2017, Burnett 2020), flight orientation (Comba 1999, Riley et al 1999) and landing abilities (Chang 2016). This study has identified an aspect of natural bee foraging behaviour not previously recognised in nature, an increase in hesitancy to take-off with increasing wind speed. Why this hesitancy occurs, we cannot yet explain. However, considering foraging bees can visit up to 20 flowers a minute (Couvillon 2015) it has the potential to significantly reduce bee flower visit rate (Hennessy et al 2020) and therefore the energetic returns of a bee during a foraging bout. Both honey bees and bumble bees walk an energetic tight rope, with individuals using about half the energy of the nectar they gather purely for foraging (Balfour et al 2021). Honey bees often do not forage on days with high wind (Tuell and Isaacs 2010). This is most likely due to the decrease in flower visits when foraging in windy conditions (Hennessy et al in press, Hennessy et al 2020) potentially making foraging unprofitable. Honey bees and bumbles are important pollinator species, responsible for the pollination services of many crop and wild plant species (Klein et al 2007, Klein et al 2018). Understanding how pollinator species respond to changes in wind speed is potentially important, especially as wind speeds are predicted to increase (Zeng et al 2019). More work on a range of pollinator species is needed to fully understand not only how wind can influence individual foraging efficiency, but also potentially the pollinator community (Tuell and Isaacs 2010, Brittain et al 2013) and its implications for pollination services.

For honey bees, it does not seem to be due to the indirect effects of wind causing an increase in flower movement, which was seen for nearly all plant species in this study. When averaged

across wind speeds, there were differences in take-off times between plant species, indicating that flower morphology can influence this behaviour independently of wind speed. Increased hesitancy and increasing wind speeds have the potential to influence the foraging efficiency of honey bees and bumble bees on a range of plant species. Future work could investigate what causes this increase in take-off time, whether it is seen in other pollinator species, and if plants located in particularly windy locations, such as along coasts, have adaptations to combat the influence of wind on visiting pollinators.

Chapter Nine: General Discussion

9.1 Population estimates of two rare solitary bee species

In Chapters Two and Three key information was identified on the foraging ecology and population sizes of two rare species of solitary bee, *Anthophora retusa* and *Eucera longicornis*. As both population size and the spatio-temporal changes in population size are some of the most important predictors in determining extinction risk, their accurate estimation is vital for conservation biology (Murray et al 2009). For *A. retusa* the population located at Seaford head is thought to be one of the strongest of the species in the UK (Edwards and Jenner 2008). However, prior to the estimates in Chapter Two there had been no quantitative assessments of the population. The results in Chapter Two indicate the male and female population was approximately 100 individuals in 2018, with an equal sex ratio, and a male population of 160 individuals in 2019. Low population sizes increase local extinction risk due both stochastic and human-mediated deterministic factors (e.g. habitat change, Frankham 2002). Low population sizes can also be of an increased risk to Hymenoptera due to their complementary sex determination system (Zayed and Packer 2005, Zayed 2009). In small populations the chances of matched matings, resulting in sterile males, is increased (Zayed 2009), which significantly increases a populations extinction risk. There was an increase in the estimated male population between 2018 and 2019. However, two years of data collection is not enough to gauge if the population is increasing in size. Due to natural variation in population growth, insect populations commonly experience large annual fluctuations (Schultz and Hammond 2003). Future work should continue to assess the size of the population to determine changes in trends, especially as the low population estimates obtained in Chapter Two indicate this population is at risk.

The population estimates of *Eucera longicornis* at Gatwick were conducted at two aggregations, and unlike the *A. retusa* population the sex ratios were not equal in the final year of surveying. This is a surprising result, especially as the ratios were female biased. Sexual dimorphism is common in the Hymenoptera, with females generally being larger (Helms 1994), making them more costly to produce. When local resource availability is good, this can result in it being beneficial to produce the more costly sex (Kim 1999) due to females being able to allocate more resources to each individual offspring (Peterson et al 2006). The female bias seen with in the *E. longicornis* aggregations may be an indication of good quality resources located nearby to the aggregations.

Population estimates using techniques such as mark recapture are useful and are often used in studies on insects and other species (Saul 1987, Larsson and Franzén 2008, Cooper et al 2015). However, they are highly time consuming and not always possible. Other appropriate methods include survey walks, which were found to be highly correlated to estimates obtained through mark recapture when used on a population of the solitary bee *Andrena hattorfiana* (Larsson and Franzén 2008). Continued monitoring of the *E. longicornis* populations is recommended to determine if the habitat management being put in place is effective.

9.2 Foraging ecology

The viability and extinction risk of a population is not only determined by the population size, but also by the quality of the habitat where it is found, along with the chances of immigration or emigration from other populations (Franzén and Nilsson 2009). To determine if a habitat is of suitable quality, the requirements of the species must be known. These include both nest and forage requirements. For *A. retusa* and *E. longicornis*, through surveys of both bees on flowers and through analysing pollen collected from females, a detailed account of forage requirements was obtained. Perhaps surprisingly, considering the rarity of the species, *A. retusa* was found to have a wide range of forage species, including the common *Glechoma hederacea* (ground ivy). It is often reported that species with a narrow diet breadth are more at risk of decline (Hofmann et al 2020). For example, in England 96 bee species have been classed as threatened or vulnerable (Falk 1991), with 29 (30%) of these pollen specialists (Pekkarinen 1998). The rarity of *A. retusa* does not seem to be due to the narrow diet breadth.

E. longicornis appears to be a species which does follow this pattern. Pollen analysis of returning females indicates the species has a narrow diet, predominantly consisting of species from the Fabaceae family. This is consistent with work on other *E. longicornis* populations (Saunders 2014), indicating it is not specific to the Gatwick aggregations. This narrow diet breadth may help explain the species' general decline. Fabaceae species are common in flower rich grasslands, which are declining in the UK (Goulson et al 2005). Between early and late twentieth century, flower rich grasslands declined by more than 55% and were replaced with permanent grassland (Ollerton et al 2014). These changes in landscape management have resulted in both a reduction in the range of many of the pollen sources *E. longicornis* rely on, as well as their frequency at a more local scale. For example, *L.*

pratensis, a major pollen source for *E. longicornis*, reduced in frequency by 45% in the UK between 1978 and 1998 (Carvell et al 2006).

However, being a specialist does not necessarily put you at risk of population or distribution decline. *Colletes hederae*, the ivy bee, has experienced a rapid range expansion across Europe since it was first identified as a species in 1991 (Dellicour et al 2014). In Chapter Four, *C. hederae* pollen samples were found to contain on average 98.5% of pollen from *Hedera*, ivy species, indicating it is highly specialised on the plant. Since its arrival in the UK in 2001, where it was first sighted in Dorset, *C. hederae* has spread as far north as Carlisle (NBN 2020). The rapid expansion of this specialist bee highlights how if the resource you specialise on is common, then being a specialist does not necessarily increase the risk of decline. *C. hederae* also emerges in late autumn when competition from other pollinator species is reduced. In Chapter Four it was found to be the most common forager on ivy plants, being even more common than the eusocial honeybee. The species is also thought to have good dispersal abilities and a high reproductive rate (Bischoff et al 2005).

Solitary bee species often have small foraging ranges, meaning they require a high density of resources per unit area (Cresswell et 2000). For *A. retusa*, although the exact foraging range could not be calculated, by using the locations of recapture events, an estimate of how far individuals travel during a foraging bout could be calculated. Most recaptures occurred within 30m, with an average distance of 122m. For *E. longicornis*, no marked individuals were caught when conducting surveys of the local habitat. However, an unmarked male was caught c. 500m away from the nearest aggregation. Both these distances indicate the importance of high-quality and abundant forage close to the nesting sites. For *E. longicornis*, both aggregations had a high abundance of Fabaceae species, their main pollen source, within 100m of the nest aggregations throughout their flight season. An abundance of good quality forage near to the nesting site helps reduce the costs of foraging and therefore potentially increases reproductive output (Zurbuchen et al 2010b), perhaps helping to explain the high female sex bias seen in the aggregations.

For *A. retusa*, the abundance of forage changed throughout its flight season. Most notably, *G. hederae* began to reduce, with species such as *Iris foetidissima*, stinking iris, and *Echium vulgare*, vipers bugloss, becoming more abundant. *A. retusa* would switch their foraging behaviour, foraging on the more abundant species. This was also seen in Chapter Four with

C. hederae. When ivy was not yet at its peak, females would collect pollen from other species. This shifting of foraging patterns is seen with many bee species (Ogilvie et al 2014).

9.3 Explanations for changes in abundance

The reasons for the decline of *E. longicornis* in the UK are most likely due to a reduction in habitat and the food plants which they specialise on. The expansion of *C. hederae* both in mainland Europe and the UK is most likely due to it foraging on a common and highly abundant plant species. So why is *A. retusa* so rare? The fact that *A. retusa* are flexible in their foraging habits indicates that perhaps nesting requirements are a limiting factor to their distribution. Some solitary bee species require specific nesting requirements, such as certain materials or substrates (Franzén et al 2007). Although the exact nest site of *A. retusa* at Seaford Head was not determined, it is most likely that they are nesting in the loess deposits on the cliff face (Edwards and Jenner 2008, Horsley et al 2013). There is little information on where they nest in the other sites where they are found. However, many of the remaining populations are coastal, with access to chalk cliffs. More research is required on their nesting requirements to determine the impact this may be having on their distribution.

Although studying individual populations of a species is important to contribute to the general knowledge of a species distribution, abundance and behaviour, there are limitations to the methods used in this thesis. By focussing on a single population of a species, conclusions regarding how the local habitat may influence aspects of behaviour, such as foraging, cannot be made. As is seen with *C. hederae* both in Chapter Four in this thesis and in previous studies (Teppner & Brosch 2015) local floral availability can influence the foraging habits of bee species. As mentioned previously, details on nesting characteristics can also not be determined from only studying individuals in one location. However, detailed information on population size can be, which may not be possible if multiple sites are studied. For *A. retusa* and *E. longicornis,* where studies on the species in the UK are rare, even single population studies are important as they widen our limited knowledge both on the abundance of the species, but also their forage and habitat requirements.

9.4 Effects of wind on foraging ecology

Chapters Two through Four provide valuable information on two rare and one common species of solitary bee. They highlight the importance of effective monitoring of populations and identifying the requirements of species to aid in conservation. However, not only is identifying habitat and forage requirements important for conservation, but also

understanding the foraging behaviour of species and how this can be influenced by the environment. Many environmental factors are known to influence both the distribution (Classen et al 2015, Kerr et al 2015) and behaviour (Vicens and Bosch 2000, Peat and Goulson 2005, Kuppler et al 2021) of bee species. Global wind speeds are currently increasing (Zeng et al 2019), and extreme weather events, including storms, are predicted to increase in frequency (Mirza 2003). Because of this changing climate, understanding how these extreme events may impact bees, such as by studying the impacts of wind speed on their foraging behaviour, is becoming more important for their conservation.

9.5 Hesitancy to take off

Previous work has shown that with increasing winds one often sees a reduction in honeybee foraging (Vicens and Bosch 2000, Tuell and Issacs 2010, Brittain 2013). However, studies examining how wind influences the foraging efficiency of bees, perhaps explaining why this reduction is seen, are few.

Increasing wind was found to significantly reduce the number of flower visits of foraging honeybee workers both when foraging on artificial flowers (Chapter Five) and on real flowers in the field (Chapters Six and Seven). Chapter Five identified the cause for this reduction; bees were more hesitant to take off from a flower in higher wind speeds, resulting in longer handling times. This is the first time the idea of 'hesitancy' has been identified in foraging bees. Previous work has shown how wind is able to alter flight mechanics and decisions made during flight. For example, Burnett et al (2020) showed that honeybees will fly faster towards moving obstacles when travelling in windy conditions, whereas they will slow down when flying in still air. Work studying how wind influences take off in insects is limited, often examining it in relation to migration events (Chapman et al 2015), or how to improve the take-off abilities of micro aerial vehicles (Nguyen et al 2016). The work in Chapter Five was completed on artificial flowers, which poses the question, does hesitancy to take off occur in nature? The results in Chapter Eight suggest it does, with hesitancy to take off increasing with wind speed for both honeybees and bumblebees when foraging on a range of plant species. The response of honeybees and bumblebees to increasing wind did not differ. This is perhaps surprising given the results in Chapter Seven, where increasing wind speed caused an increase in handling time for honeybees foraging on lavender, but no effect on bumblebees. This may be due to the wind speeds experienced in Chapter Seven, which only reached a maximum speed of $3.56ms^{-1}$, compared to $6.5ms^{-1}$ in Chapter Eight. Perhaps, due to

bumblebee species' larger size, it requires higher wind speeds for effects to be seen (Hunter 2007).

In Chapter Five, the increase in hesitancy resulted in a reduction in flower visitation rate. This reduction in flower visits potentially reduces the amount of nectar an individual bee can collect in a given unit of time. Bees make foraging decisions based on profitability (Cnaani et al 2006, Balfour et al 2021), so this reduction in flower visits may help explain why honeybees tend not to forage in high winds speeds. Again, however, this reduction was seen when honeybees were foraging on artificial flowers. Would the same result be true in nature on real flowers? When foraging on lavender and marjoram honeybee handling time per flower significantly increased with increasing wind speed and this increase resulted in a significant reduction in flower visitation rate. Handling time per flower included all behaviours which occur on the flower (take off, collecting pollen or nectar as well as walking between flowers). Hesitancy to take off appears to be an important component of the foraging process, capable of significantly influencing the foraging efficiency of bees.

9.6 Indirect effects of wind

When comparing honeybees foraging on lavender and marjoram, flower visit rate significantly decreased with increasing wind speed for both species. However, there was a difference in the extent of the reduction. Independently of wind speed, differences in flower morphology are capable of influencing visitation rate; for example, the length of the corolla tube (Inouye 1980, Balfour et al 2013), nectar concentration (Mallinger and Prasifka 2017) and flower density (Ohashi and Yahara 2002). However, how these variables interact with wind to influence foraging efficiency is unknown. Not only is wind capable of influencing foraging behaviour directly, but also indirectly via the movement of flowers. In Chapter Five the direct and indirect effects of wind were separated, with flower movement found to have no significant effect on flower visitation rate. This result suggests that when working independently, wind speed has more of an impact on foraging rate than flower movement. However, in nature it is unlikely a bee would experience one without the other. Therefore, how does the interaction between flower movement and wind influence foraging behaviour? The extent of movement is dependent on the plant species due to differing plant morphology (Chapters Six and Eight, Yamazaki 2011, Warren and James 2008). For example, in Chapter Eight the plant species which was found to move the most in wind was *Jacobaea vulgaris* (ragwort), most likely due to its long stems and top-heavy flowers. Lavender flowers were

also found to move more with increasing wind speed compared to marjoram, and when examining the effects of this flower movement it was found to not influence flower visitation rate. Previous studies examining flower movement, and its effects on insect foraging behaviour, have found it can be beneficial, both in relation to the insect and the plant. For example, increased leaf movement is thought to be beneficial by reducing herbivory and damage by pathogens (Yamazaki 2011 and Warren 2015). For insects, moderate flower mobility has been found to attract more pollinators compared to less mobile flowers or those with high mobility (Warren and James 2008). This increased attraction may be due to moving flowers being easier to detect, with honeybees previously shown to choose moving artificial flowers verses stationary ones (Wolf & Zerrahn-Wolf 1937). When foraging on moving artificial flowers, in Chapter Five, honeybees did spend less time searching for the nectary once they had landed. This may have been due to increased landing accuracy on mobile vs stationary flowers, as has been seen with bumblebees (Kapustjansky et al 2010).

Natural flower movement in wind was also found not to influence hesitancy to take off in honeybees and bumblebees. However, bumblebees' hesitancy did increase more with increasing flower movement when compared with honeybees (Chapter Eight). This difference in response between bumblebees and honeybees, in relation to flower movement, may be due to their abilities to grip. Both bumblebees and honeybee have tarsal claws which aid in gripping to surfaces. Due to bumblebees' larger body size, they have blunter claws, which provide less grip compared to those of a honeybee (Pattrick 2018). The difficulties in gripping onto a flower surface can alter flower choice in bumblebees. Foraging *Bombus terrestris* were shown to choose flowers with conical shaped cells on their surface, which provide better grip, over flowers with smoother surfaces when flowers were moving (Whitney et al 2009), indicating lack of grip is potentially detrimental to foraging efficiency.

9.7 Future research ideas

This thesis has attempted to increase our understanding of wind and how it influences the foraging behaviour and ability of honeybees and bumblebees. It is an environmental variable highly capable of not only influencing flight mechanics (Pinzauti 1986, Comba 1999, Crall et al 2017, Ravi et al 2013) but also their foraging behaviour and efficiency. Considering that wind speeds are projected to increase, more work is needed to fully understand the complex interactions between pollinators, wind, and plants. Future work could address flower morphology, such as the structure of petals, and how this influences species abilities to forage

in windy conditions. Work on how other pollinator species respond to wind is also needed, as their responses could be very different. Some species continue to forage in windy conditions, even once honeybees have stopped (Brittain et al 2013). This reduction in honeybees has been found to influence the foraging behaviour of the still present other species (Tuell and Issac 2010, Brittain et al 2013) perhaps due to a release of competition as honeybees and bumblebees are capable of outcompeting solitary bee species (Wignall et al 2020). Understanding how wind, and other environmental variables, influence the pollinator community is an area of research worth investigating.

Conclusion

This thesis has contributed novel knowledge on the foraging ecology of three solitary bee species along with how an understudied environmental variable, wind, influences foraging behaviour of both honey bees and bumble bees. Information from the studies on *A. retusa* and *E. longicornis* are currently being used by landowners to ensure their land is managed in such a way as to help conserve the populations of these species located on their property. Chapter Four is the first study of *C. hederae* and its foraging ecology in England and provides important information regarding a non-native species and its potential interactions with our native pollinators. Although less directly linked with conservation, understanding how our bee species' foraging may be influenced by wind is becoming more important with future climate predictions of increasing wind speeds and storm events, with work from this thesis identifying the novel behaviour of hesitancy to take off in wind. The work from this thesis has contributed novel research and findings both to the area of solitary bee conservation and to bee foraging behaviour and ecology.

References

Ahrne K, Bengtsson J, & Elmqvist T (2009) Bumble bees (*Bombus* spp) along a gradient of increasing urbanization. PloS one https://doi.org/10.1371/journal.pone.0005574

Alcorn, K., Whitney, H. and Glover, B. (2012) Flower movement increases pollinator preference for flowers with better grip. Functional Ecology, *26*(4), 941-947

Alma, A.M., Farji-Brener, A.G. and Elizalde, L. (2016) Gone with the wind: short-and long-term responses of leaf-cutting ants to the negative effect of wind on their foraging activity. Behavioral Ecology, *27*(4), 1017- 1024

Alma, A. M., Farji-Brener, A. G., & Elizalde, L. (2017). A breath of fresh air in foraging theory: the importance of wind for food size selection in a central-place forager. The American Naturalist, *190*(3*)*, 410-419.

Alves-dos-Santos, I. (2003). Trap-nesting bees and wasps on the university campus in Sao Paulo, Southeastern Brazil (Hymenoptera: Aculeata). Journal of the Kansas Entomological Society, *76*(2),328-334.

Arbuthnott, D., & Brigham, R. M. (2007). The influence of a local temperature inversion on the foraging behaviour of big brown bats, *Eptesicus fuscus*. Acta Chiropterologica, *9(1)*, 193-201.

Aubert M. (2013) *Anthophora retusa*. https://www.bwars.com/bee/apidae/anthophora-plumipes date accessed December 12 2019.

Aublet, J.F., Festa-Bianchet, M., Bergero, D. and Bassano, B. (2009) Temperature constraints on foraging behaviour of male Alpine ibex (*Capra ibex*) in summer. Oecologia, *159*(1), 237-247.

Åkesson, S., & Hedenström, A. (2007). How migrants get there: migratory performance and orientation. *BioScience*, *57*(2), 123-133.

Balfour, N. J., Garbuzov, M., & Ratnieks, F. L. (2013). Longer tongues and swifter handling: why do more bumble bees (*Bombus* spp.) than honey bees (*Apis mellifera*) forage on lavender (*Lavandula* spp.) Ecological entomology, *38*(4), 323-329.

Balfour, N. J., Gandy, S., & Ratnieks, F. L. (2015). Exploitative competition alters bee foraging and flower choice. Behavioral Ecology and Sociobiology, *69*(10), 1731-1738.

Balfour, N. J., Shackleton, K., Arscott, N. A., Roll-Baldwin, K., Bracuti, A., Toselli, G., & Ratnieks, F. L. (2021). Energetic efficiency of foraging mediates bee niche partitioning *102*(4): e03285. 10. 1002/ecy.3285.

Baldock, K. C., Goddard, M. A., Hicks, D. M., Kunin, W. E., Mitschunas, N., Osgathorpe, L. M., ... & Memmott, J. (2015). Where is the UK's pollinator biodiversity? The importance of urban areas for flower-visiting insects. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1803), 20142849.

Baldock K., Goddard M. A., Kunin W. E., Potts S. G., Stone G. N., & Memmott J. (2015) Managing urban areas for insect pollinators: As town and cities continue to grow how can land managers help insect pollinators in urban areas? Accessed from https://research-information.bris.ac.uk/files/52299185/LWEC_20_PPN.pdf Accessed 02.03.20

Banaszak-Cibicka W. & Żmihorski M. (2012) Wild bees along an urban gradient: winners and losers. Journal of Insect Conservation, *16*(3), 331-343

Barnett, L. K., & Warren. (1995). High Brown Fritillary *Argynnis Adippe*. Butterfly Conservation.

Batra S. W. (1978) Aggression, territoriality, mating and nest aggregation of some solitary bees (Hymenoptera: Halictidae, Megachilidae, Colletidae, Anthophoridae). Journal of the Kansas Entomological Society, *51*(4) 547-559.

Batra, S. W. (1984). Solitary bees. Scientific American, *250*(2), 120-127.

BBC. (2017) *Second search at Seaford Head after further cliff fall* https://www.bbc.co.uk/news/uk-england-sussex-40371106 Date accessed December 19 2019

Beebee, T. J., & Griffiths, R. A. (2005) The amphibian decline crisis: a watershed for conservation biology?. Biological Conservation, 125(3), 271-285

Bees, Wasps & Ants Recording Soceity (2012) *Eucera longicornis.*: https://www.bwars.com/bee/apidae/eucera-longicornis Date accessed 1 February 2020.

Bees, Wasps & Ants Recording Society, (2013) *Anthophora retusa*. http://www.bwars.com/index.php?q=bee/apidae/anthophora-retusa. Date accessed January 8 2020

Bees, Wasps & Ants Recording Society, (2016), *Hoplitis adunca*, https://www.bwars.com/bee/megachilidae/hoplitis-adunca. Date accessed 24 February 2021

Bees, Wasps & Ants Recording Society, (2018), *Colletes hederae* http://www.bwars.com/bee/colletidae/colletes-hederae. Date accessed 12 December 2018

Berlincourt, M., & Arnould, J. P. (2015). Influence of environmental conditions on foraging behaviour and its consequences on reproductive performance in little penguins. Marine biology, *162*(7), 1485-1501.

Biesmeijer, J. C., Roberts, S. P., Reemer, M., Ohlemüller, R., Edwards, M., Peeters, T., & Settele, J. (2006) Parallel declines in pollinators and insect-pollinated plants in Britain and the Netherlands. Science, *313*(5785), 351-354.

Biological Records Centre. *Glechoma hederacea*. https://www.brc.ac.uk/plantatlas/plant/glechoma-hederacea date accessed 15 June 2020

Bischoff I (2003) Population dynamics of the solitary digger bee *Andrena vaga Panzer* (Hymenoptera, Andrenidae) studied using mark-recapture and nest counts. Population Ecology, *45*(3), 197-204

Bischoff, I., Eckelt, E. and Kuhlmann, M., (2005) On the biology of the ivy-bee *Colletes hederae* Schmidt & Westrich, 1993 (Hymenoptera, Apidae). Bonner Zoologische Beiträge, *53*(2004), 27-36.

Blazyte-Cereskiene L, Vaitkeviciene G, Venskutonyte S, Buda V. (2010) Honey bee foraging in spring oil seed rape crops under high ambient temperature conditions. Zemdirb Agric, *97*(2010),61–70.

Bodsworth, E., Shepherd, P. and Plant, C. (2005). Exotic plant species on brownfield land: their value to invertebrates of nature conservation importance. English Nature Resources Report No. 650.

Boyd, I.L., McCafferty, D.J. and Walker, T.R. (1997) Variation in foraging effort by lactating Antarctic fur seals: response to simulated increased foraging costs. Behavioral Ecology and Sociobiology, *40*(3), 135-144

Brittain, C. A., Vighi, M., Bommarco, R., Settele, J., & Potts, S. G. (2010). Impacts of a pesticide on pollinator species richness at different spatial scales. Basic and Applied Ecology, *11*(2), 106-115.

British Airport Authority (2004) River mole diversion fish monitoring.

Brown, M. J., & Paxton, R. J. (2009). The conservation of bees: a global perspective. Apidologie, *40*(3), 410-416.

Brown, A., Gilbert, G., & Wotton, S. (2012) Bitterns and bittern conservation in the UK. British Birds, *105*(2), 58

Buchmann, S., & Ascher, J. S. (2005) The plight of pollinating bees. Bee World, 86(3).

Buglife (2009) Planning for brownfield biodiversity: a best practice guide. Buglife – The Invertebrate Conservation Trust, Peterborough.

Burnham K. P., & Anderson, D. R. (1998) Practical use of the information-theoretic approach. In Model selection and inference (pp. 75-117). Springer, New York, NY.

Burnett, N. P., Badger, M. A., & Combes, S. A. (2020). Wind and obstacle motion affect honeybee flight strategies in cluttered environments. *Journal of Experimental Biology*, *223*(14) jeb222471

Butterfly Conservation Trust. *Duke of Burgundy*. https://butterfly-conservation.org/butterflies/duke-of-burgundy date accessed January 8 2020

Cameron, S. A., Lozier, J. D., Strange, J. P., Koch, J. B., Cordes, N., Solter, L. F., & Griswold, T. L. (2011) Patterns of widespread decline in North American bumble bees. P. Natl. A. Sci., *108*(2), 662-667

Cane, J. H., & Sipes, S. (2006) Characterizing floral specialization by bees: analytical methods and a revised lexicon for oligolecty. Plant-pollinator interactions: from specialization to generalization, *99*(2006), 99-122.

Cane, J. H., Minckley, R. L., Kervin, L. J., Roulston, T. A. H., & Williams, N. M. (2006). Complex responses within a desert bee guild (Hymenoptera: Apiformes) to urban habitat fragmentation. *Ecological Applications*, *16*(2), 632-644.

Cane, J. H. (2020). A brief review of monolecty in bees and benefits of a broadened definition. Apidologie, *52*, 77-22.

Cannell, B.L. and Cullen, J.M. (1998) The foraging behaviour of little penguins *Eudyptula minor* at different light levels. Ibis, *140*(3), 467-471

Carvell C., Roy D. B., Smart S. M., Pywell R. F., Preston C. D., & Goulson D. (2006) Declines in forage availability for bumblebees at a national scale. Biological Conservation, *132*(4), 481-489

Centre for Ecology and Hydrology. (2008). *Large Blue Butterfly.* https://www.ceh.ac.uk/large-blue-butterfly. Date Accessed 25 February 2021

Chang, J.J., Crall, J.D. and Combes, S.A. (2016). Wind alters landing dynamics in bumblebees. Journal of Experimental Biology, *219*(18), 2819-2822

Chevalier S., & Cohen J. R. (1997) *Twenty-eight Year Study of Upland Sandpiper Breeding* Population. https://sora.unm.edu/sites/default/files/journals/nabb/v022n03/p0109-p0113.pdf Date accessed 4 March 2020.

Chittka, L., Gumbert, A., & Kunze, J. (1997) Foraging dynamics of bumble bees: correlates of movements within and between plant species. Behavioral Ecology, *8*(3), 239-249.

Chittka, L., Dyer, A.G., Bock, F. and Dornhaus, A. (2003) Psychophysics: bees trade off foraging speed for accuracy. Nature, 424(6947), 388

Church, N. S. (1960). Heat loss and the body temperatures of flying insects: II. Heat conduction within the body and its loss by radiation and convection. Journal of Experimental Biology, *37*(1), 186-212.

Civil Aviation Authority (2020) *UK airport data.* https://www.caa.co.uk/Data-and-analysis/UK-aviation-market/Airports/Datasets/UK-airport-data/ date accessed 30 April 2020.

Classen, A., Peters, M. K., Kindeketa, W. J., Appelhans, T., Eardley, C. D., Gikungu, M. W., ... & Steffan-Dewenter, I. (2015). Temperature versus resource constraints: which factors determine bee diversity on M ount K ilimanjaro, T anzania?. *Global Ecology and Biogeography*, *24*(6), 642-652.

Clark, J.R. (1983) Age-related changes in trees. Journal of Arboriculture, *9*(8), 201– 205.

Cnaani, J., Thomson, J. D., & Papaj, D. R. (2006). Flower choice and learning in foraging bumblebees: effects of variation in nectar volume and concentration. Ethology, *112*(3), 278-285.

Comba, L. (1999) Patch use by bumblebees (Hymenoptera Apidae): temperature, wind, flower density and traplining. Ethology Ecology and Evolution, 11(3), 243-264

Combes, S.A. and Dudley, R. (2009) Turbulence-driven instabilities limit insect flight performance. Proceedings of the National Academy of Sciences, *106*(22), 9105-9108

Conradt, L., Clutton-Brock, T. H., & Guinness, F. E. (2000). Sex differences in weather sensitivity can cause habitat segregation: red deer as an example. Animal Behaviour, *59*(5), 1049-1060

Cooch, E G, & White G C. (2004) Program MARK: analysis of data from marked individuals. A Gentle Introduction.

Cooper, R., Wang, C., & Singh, N. (2015). Mark-release-recapture reveals extensive movement of bed bugs (Cimex lectularius L.) within and between apartments. *PloS one*, *10*, e0136462.

Couvillon, M. J., Schürch, R., & Ratnieks, F. L. (2014). Waggle dance distances as integrative indicators of seasonal foraging challenges. PloS one, *9*, e93495.

Couvillon, M.J., Walter, C.M., Blows, E.M., Czaczkes, T.J., Alton, K.L. and Ratnieks, F.L. (2015) Busy bees: variation in insect flower-visiting rates across multiple plant species. Psyche, 2015:134630.

Crall, J. D., & Combes, S. A. (2013, April). Blown in the wind: Bumblebee temporal foraging patterns in naturally varying wind conditions. Integrative and Comparative Biology *53*, 270-E270.

Crall, J.D., Chang, J.J., Oppenheimer, R.L. and Combes, S.A. (2017) Foraging in an unsteady world: bumblebee flight performance in field-realistic turbulence. Interface focus, *7*(1), 20160086

Cross, I. C. (2002) *Colletes hederae* Schmidt & Westrich (Hym., Apidae) new to mainland Britain with notes on its ecology in Dorset. Entomologists Monthly Magazine, *138*, 201-203.

Croxton, P. J., Hann, J. P., Greatorex-Davies, J. N., & Sparks, T. H. (2005) Linear hotspots? The floral and butterfly diversity of green lanes. Biological Conservation, *121*(4), 579-584

Crowther, L. (2017). *The Tree Bumblebee, Bombus hypnorum: ecology and genetics of a naturally colonising pollinator* (Doctoral dissertation, University of East Anglia).

Danforth, B. N., Minckley, R. L., Neff, J. L., & Fawcett, F. (2019). *The solitary bees: biology, evolution, conservation*. Princeton University Press.

Davies, C. R., & Gilbert, N. (1985) A comparative study of the egg-laying behaviour and larval development of *Pieris rapae* L. and *P. brassicae* L. on the same host plants. *Oecologia*, *67*(2), 278-281.

Davis, H. K., Miller, D. L., & Thetford, M. (2020) Habitat suitability of an at-risk, monolectic, ground-nesting bee *Hesperapis oraria* and its floral host *Balduina angustifolia* at two spatial scales along the Northern Gulf of Mexico. Journal of Insect Conservation, *24*(3), 561-573.

De Knegt, H.J., Hengeveld, G.M., Van Langevelde, F., De Boer, W.F. and Kirkman, K.P. (2007) Patch density determines movement patterns and foraging efficiency of large herbivores. Behavioral Ecology, *18*(6), 1065-1072

Dellicour, S., Mardulyn, P., Hardy, O. J., Hardy, C., Roberts, S. P. M., & Vereecken, N. J. (2014) Inferring the mode of colonization of the rapid range expansion of a solitary bee from multilocus DNA sequence variation. Journal of evolutionary biology, *27*(1), 116-132.

De Langre, E. (2008). Effect of wind on plants. Annual Review of Fluid Mechanics, *40*, 141– 168.

De Manincor, N., Hautekèete, N., Mazoyer, C., Moreau, P., Piquot, Y., Schatz, B., ... & Massol, F. (2020). How biased is our perception of plant-pollinator networks? A comparison of visit-and pollen-based representations of the same networks. Acta Oecologica, *105*, 103551.

De Palma, A., Abrahamczyk, S., Aizen, M. A., Albrecht, M., Basset, Y., Bates, A., ... & Purvis, A. (2016). Predicting bee community responses to land-use changes: Effects of geographic and taxonomic biases. Scientific Reports, *6*(1), 1-14.

De Pascalis, F., Imperio, S., Benvenuti, A., Catoni, C., Rubolini, D., & Cecere, J. G. (2020). Sex-specific foraging behaviour is affected by wind conditions in a sexually size dimorphic seabird. Animal Behaviour, *166*, 207-218.

Didham R K, Basset Y, Collins C M, Leather S R,…Stewart, A.J. (2020) Interpreting insect declines: seven challenges and a way forward. Insect Conservation and Diversity, 13(2), 103-114

Digby P.S. (1955) Factors affecting the temperature excess of insects in sunshine. Journal of Experimental Biology, *32*(2), 279-289

Ditner, N., Balmer, O., Beck, J., Blick, T., Nagel, P., & Luka, H. (2013). Effects of experimentally planting non-crop flowers into cabbage fields on the abundance and diversity of predators. *Biodiversity and conservation*, *22*(4), 1049-1061.

Downing, J., Borrero, H., & Liu, H. (2016). Differential impacts from an extreme cold spell on subtropical vs. tropical specialist bees in southern Florida. *Ecosphere*, *7*(5): e01302.

Dunn, R. R. (2005) Modern insect extinctions, the neglected majority. Conservation Biology, *19*(4), 1030-1036

Edwards, M. & Jenner, M. (2008) *Anthophora retusa: the Potter Flower Bee. Hymettus.* Available at http://hymettus.org.uk/downloads/A%20retusa%202008.pdf date accessed 19 August 2020.

Edwards, E. K., Mitchell, N. J., & Ridley, A. R. (2015). The impact of high temperatures on foraging behaviour and body condition in the Western Australian Magpie *Cracticus tibicen* dorsalis. Ostrich, *86*(1-2), 137-144.

Eickwort, G. C., & Ginsberg, H. S. (1980) Foraging and mating behaviour in Apoidea. Annual Review of Entomology, *25*(1), 421-446

Elliott, K. H., Duffe, J., Lee, S. L., Mineau, P., & Elliott, J. E. (2006). Foraging ecology of Bald Eagles at an urban landfill. The Wilson Journal of Ornithology, *118*(3), 380-390.

Ellis, S., Wainwright, D., Dennis, E.B., Bourn, N.A.D., Bulman, C.R., Hobson, R., Jones, R., Middlebrook, I., Plackett, J., Smith, R.G. and Wain, M. (2019) Are habitat changes driving the decline of the UK's most threatened butterfly: the High Brown Fritillary *Argynnis adippe* (Lepidoptera: Nymphalidae)? Journal of Insect Conservation, *23*(2), 351-367.

Erni, B., Liechti, F., Underhill, L. G., & Bruderer, B. (2002). Wind and rain govern the intensity of nocturnal bird migration in central Europe-a log-linear regression analysis. *Ardea*, *90*(1), 155-166.

Esch, H. (1988) The effects of temperature on flight muscle potentials in honeybees and cuculiinid winter moths. Journal of Experimental Biology, 135(1), 109-117

Evans R.L. & Potts S.G. (2004) Iconic bees: South East England. Potter Flower Bee. Friends of the Earth.

Falk S. (1991) A review of the scarce and threatened bees, wasps and ants of Great Britain (No. 35). Nature Conservancy Council for England.

Falk S, & Lewington R (2015) Field Guide to the Bees of Great Britain and Ireland. London, Bloomsbury Publishing

Fellendorf, M., Mohra, C., & Paxton, R. J. (2004). Devasting effects of river flooding to the ground-nesting bee, Andrena vaga (Hymenoptera: Andrenidae), and its associated fauna. *Journal of Insect Conservation*, *8*(4), 311-312.

Fisher, R. A. (1930) The genetical theory of natural selection. Clarendon, Oxford.

Formstone B, & Howe M. (2013) Brownfield sites and their value for invertebrates–A survey of selected sand quarries in north-east Wales: Borras Quarry and Marford Quarry.

Forrest, J. R., & Chisholm, S. P. (2017). Direct benefits and indirect costs of warm temperatures for high-elevation populations of a solitary bee. *Ecology*, *98*,(2) 359-369.

Fowler, R. E., Rotheray, E. L., & Goulson, D. (2016). Floral abundance and resource quality influence pollinator choice. *Insect Conservation and Diversity*, *9*(6), 481-494.

de Oliveira Francisco, F., & Arias, M. C. (2010). Inferences of evolutionary and ecological events that influenced the population structure of *Plebeia remota*, a stingless bee from Brazil. Apidologie, *41*(2), 216-224.

Frankham, R., Ballou, J. D., & Briscoe, D. A. (2002). Conservation genetics.

Franzén, M., Larsson, M., & Nilsson, S. G. (2009) Small local population sizes and high habitat patch fidelity in a specialised solitary bee. Journal of Insect Conservation, *13*(1), 89-95

Franzén, M. & Nilsson, S. G. (2010) Both population size and patch quality affect local extinctions and colonizations. Proceedings of Royal Society B: Biological Sciences, 277(1678), 79-85

Fraser, N.H., Metcalfe, N.B., Heggenes, J. and Thorpe, J.E. (1995) Low summer temperatures cause juvenile Atlantic salmon to become nocturnal. Canadian Journal of Zoology, *73*(3), 446-451

Frechen, M. (2011). Loess in Europe. Quaternary Sci. Journal 60:3-5.

Free, J. B. (1963). The flower constancy of honeybees. The Journal of Animal Ecology, *32*(1), 119-131.

Garbuzov, M. and Ratnieks, F.L. (2014) Ivy: an underappreciated key resource to flower-visiting insects in autumn. Insect Conservation and Diversity, *7*(1), 91-102

Gathmann, A., Greiler, H. J., & Tscharntke, T. (1994). Trap-nesting bees and wasps colonizing set-aside fields: succession and body size, management by cutting and sowing. *Oecologia*, *98*(1), 8-14.

Gathmann A, & Tscharntke T (2002) Foraging ranges of solitary bees. Journal of Animal Ecology, *71*(5), 757-764

Giannini, T. C., Acosta, A. L., Garófalo, C. A., Saraiva, A. M., Alves-dos-Santos, I., & Imperatriz-Fonseca, V. L. (2012). Pollination services at risk: bee habitats will decrease owing to climate change in Brazil. Ecological Modelling, *244*, 127-131.

Gilchrist, H.G., Gaston, A.J. and Smith, J.N. (1998). Wind and prey nest sites as foraging constraints on an avian predator, the glaucous gull. Ecology, *79*(7), 2403-2414

González-Varo, J. P., Ortiz-Sanchez, F. J., & Vilà, M. (2016) Total bee dependence on one flower species despite available congeners of similar floral shape. PloS one, *11*(9), e0163122

Goodwin, R.M., Cox, H.M., Taylor, M.A., Evans, L.J. and McBrydie, H.M. (2011) Number of honey bee visits required to fully pollinate white clover (*Trifolium repens*) seed crops in Canterbury, New Zealand. New Zealand journal of crop and horticultural science, *39*(1), 7-19

Goulson, D. (1999) Foraging strategies of insects for gathering nectar and pollen, and implications for plant ecology and evolution. Perspectives in Plant Ecology, evolution and systematics, *2*(2), 185-209

Goulson D., Hanley M. E., Darvill B., Ellis J. S., & Knight M. E. (2005) Causes of rarity in bumblebees. Biological Conservation, *122*(1), 1-8

Goulson D., Lye, G. C., & Darvill B. (2008) Decline and conservation of bumble bees. Annual Review of Entomology, *53*,191-208.

Goulson, D., Nicholls, E., Botías, C. and Rotheray, E.L. (2015) Bee declines driven by combined stress from parasites, pesticides, and lack of flowers. Science, *347*(6229), 1255957

Goulson, D., & Nicholls, E. (2016). The canary in the coalmine; bee declines as an indicator of environmental health. *Science Progress*, *99*(3), 312-326.

Graham, K. K., Eaton, K., Obrien, I., & Starks, P. T. (2019). *Anthidium manicatum*, an invasive bee, excludes a native bumble bee, *Bombus impatiens*, from floral resources. Biological Invasions, *21*(4), 1089-1099.

Grant, V. (1950) The flower constancy of bees. Botanical Review, *16*(7)**,** 379-398.

Greenleaf, S. S., & Kremen, C. (2006) Wild bee species increase tomato production and respond differently to surrounding land use in Northern California. Biological Conservation, *133*(1), 81-87

Greenleaf, S. S., Williams, N. M., Winfree, R., & Kremen, C. (2007) Bee foraging ranges and their relationship to body size. Oecologia, *153*(3), 589-596

Grüter, C., Moore, H., Firmin, N., Helanterä, H. and Ratnieks, F.L. (2011) Flower constancy in honey bee workers (*Apis mellifera*) depends on ecologically realistic rewards. Journal of Experimental Biology, *214*(8), 1397-1402

Haine, E. (1955) Aphid take-off in controlled wind speeds. Nature, *175*(4454), 474-475

Hallmann, C. A., Sorg, M., Jongejans, E., Siepel, H., Hofland, N., Schwan, H., ... & de Kroon, H. (2017). More than 75 percent decline over 27 years in total flying insect biomass in protected areas. PloS one, *12*(10), e0185809.

Hampshire biodiversity action plant (2000) https://documents.hants.gov.uk/biodiversity/HampshireBiodiversityActionPlanVolume2.pdf date accessed 29 November 2020.

Harder, L. D. (1985). Morphology as a predictor of flower choice by bumble bees. Ecology, *66*(1), 198-210.

Hartig F., (2020). DHARMa: Residual Diagnostics for Hierarchical (Multi-Level / Mixed) Regression Models. R package version 0.3.3.0. https://CRAN.R-project.org/package=DHARMa

Hegland SJ, Boeke L (2006) Relationships between the density and diversity of floral resources and flower visitor activity in a temperate grassland community. Ecol Entomol *31*(5),532–538

Heinrich, B. (1975) Energetics of pollination. Annual review of ecology and systematics, *6*(1), 139-170

Heinrich, B. (1976) The foraging specializations of individual bumblebees. Ecological Monographs, *46*(2), 105-128

Heinrich, B. (1979). Resource heterogeneity and patterns of movement in foraging bumblebees. *Oecologia*, *40*(3), 235-245.

Heinrich, B. (1993) Hot-blooded Insects: Strategies and Mechanisms of Thermoregulation. Harvard University Press, Cambridge.

Heinrich, B. (1996). How the honey bee regulates its body temperature. Bee World, *77*(3), 130-137.

Helms K. R. (1994) Sexual size dimorphism and sex ratios in bees and wasps. American Naturalist, *143*(3), 418-434

Hennessy G., Harris C., Wright P., Jackson E., Goulson D., Ratnieks F.L.W. (2020) Gone with the wind: effects of wind on honey bee visit rate and foraging behaviour. Animal behaviour *161*,23-31.

Henry, M., & Rodet, G. (2018). Controlling the impact of the managed honeybee on wild bees in protected areas. Scientific reports, *8*(1), 1-10.

Herbertsson, L., Lindström, S. A., Rundlöf, M., Bommarco, R., & Smith, H. G. (2016). Competition between managed honeybees and wild bumblebees depends on landscape context. Basic and Applied Ecology, *17*(7), 609-616.

Herre E. A. (1985) Sex ratio adjustment in fig wasps. Science, *228*(4701), 896-898

Hickman, J. M., Lövei, G. L., & Wratten, S. D. (1995) Pollen feeding by adults of the hoverfly *Melanostoma fasciatum* (Diptera: Syrphidae). New Zealand Journal of Zoology, *22*(4), 387-392.

Hofmann M. M., Fleischmann A., & Renner S. S. (2020) Foraging distances in six species of solitary bees with body lengths of 6 to 15 mm, inferred from individual tagging, suggest 150m rule of thumb for flower strip distances. Journal of Hymenoptera Research, *77*,105.

Holzschuh, A., Dudenhöffer, J. H., & Tscharntke, T. (2012) Landscapes with wild bee habitats enhance pollination, fruit set and yield of sweet cherry. Biological Conservation, *153*,101-107.

Horsley, C., Whitehouse, A., & Falk, S. (2013) South West Bees Project.

Hosking, J.S., MacLeod, D., Phillips, T., Holmes, C.R., Watson, P., Shuckburgh, E.F. and Mitchell, D. (2018) Changes in European wind energy generation potential within a 1.5° C warmer world. Environmental Research Letters, *13*(5), 054032

Howard, D. C., Watkins, J. W., Clarke, R. T., Barnett, C. L., & Stark, G. J. (2003). Estimating the extent and change in broad habitats in Great Britain. Journal of Environmental Management, *67*(3), 219-227.

Hudewenz, A., & Klein, A. M. (2015). Red mason bees cannot compete with honey bees for floral resources in a cage experiment. Ecology and Evolution, *5*(21), 5049-5056.

Hunter, P. (2007). The nature of flight: the molecules and mechanics of flight in animals. EMBO reports, *8*(9), 811-813.

Hunter, P. (2014). Brown is the new green: Brownfield sites often harbour a surprisingly large amount of biodiversity. EMBO reports, *15*(12), 1238-1242.

Hunter, J. (2015). The Classic Guide to Bee Keeping: From Hives to Honey. Amberley Publishing Ltd, UK, Gloucestershire.

Hurst, M. D., Rood, D. H., Ellis, M. A., Anderson, R. S., & Dornbusch, U. (2016) Recent acceleration in coastal cliff retreat rates on the south coast of Great Britain. Proceedings of the National Academy of Sciences, *113*(47), 13336-13341

Iacobucci, D., Schneider, M. J., Popovich, D. L., & Bakamitsos, G. A. (2016). Mean centering helps alleviate “micro” but not “macro” multicollinearity. Behavior research methods, *48*(4), 1308-1317.

Inouye, D. W. (1980). The effect of proboscis and corolla tube lengths on patterns and rates of flower visitation by bumblebees. *Oecologia*, *45*(2), 197-201.

Inouye, D. W. (2020). Effects of climate change on alpine plants and their pollinators. Annals of the New York Academy of Sciences, *1469*(1), 26-37.

Inoue, T., Salmah, S., Abbas, I., & Yusuf, E. (1985). Foraging behavior of individual workers and foraging dynamics of colonies of three Sumatran stingless bees. Researches on Population Ecology, *27*(2), 373-392.

Isaacs, R., Willis, M.A. and Byrne, D.N. (1999) Modulation of whitefly take-off and flight orientation by wind speed and visual cues. Physiological Entomology, *24*(4), 311-318

Jackson, L. (2019) East of England Bee Report: A report on the status of threatened bees in the region with recommendations for conservation action. Buglife - The Invertebrate Conservation Trust, Peterborough.

Jakobi, T., Kolomenskiy, D., Ikeda, T., Watkins, S., Fisher, A., Liu, H. and Ravi, S. (2018) Bees with attitude: the effect of gusts on flight dynamics. arXiv preprint arXiv:1802.03580.

JNCC (2007) *UK Biodiversity Action Plan, Terrestrial* Invertebrateshttp://data.jncc.gov.uk/data/98fb6dab-13ae-470d-884b-7816afce42d4/UKBAP-priority-terrestrial-invertebrates.pdf date accessed 29 April 2020

JNCC (2020), *Latest UK butterfly statistics from UK Butterfly Monitoring Scheme https*://jncc.gov.uk/news/latest-uk-butterfly-statistics-from-uk-butterfly-monitoring-scheme/. Data Accessed March 1st 2020

Johnson, M. D. (1984) The pollen preferences of *Andrena (Melandrena) dunningi* Cockerell (Hymenoptera: Andrenidae). Journal of the Kansas Entomological Society, *57*(1)**,** 34-43.

Jones N. (2013) *Anthophora plumipies* female. https://www.bwars.com/bee/apidae/anthophora-plumipes date accessed 12 December 2019

Joshi, N. C., & Joshi, P. C. (2010). Foraging behaviour of *Apis* spp. on apple flowers in a subtropical environment. New York Science Journal, *3*(3), 71-76.

Kapustjansky, A., Chittka, L. and Spaethe, J. (2010) Bees use three-dimensional information to improve target detection. Naturwissenschaften, *97*(2), 229-223

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., ... & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, *349*(6244), 177-180.

Kershner E. L., & Bollinger E. K. (1996) Reproductive success of grassland birds at east-central Illinois airports. American Midland Naturalist, *136*(2),358-366.

Kéry, M., Matthies, D., & Fischer, M. (2001) The effect of plant population size on the interactions between the rare plant *Gentiana cruciata* and its specialized herbivore *Maculinea rebeli*. Journal of Ecology, *89*(3), 418-427.

Kharouba, H. M., Ehrlén, J., Gelman, A., Bolmgren, K., Allen, J. M., Travers, S. E., & Wolkovich, E. M. (2018). Global shifts in the phenological synchrony of species interactions over recent decades. Proceedings of the National Academy of Sciences, *115*(20), 5211-5216.

Kim, J. Y. (1999). Influence of resource level on maternal investment in a leaf-cutter bee (Hymenoptera: Megachilidae). Behavioral Ecology, 10(5), 552-556

Kleijn, D., Winfree, R., Bartomeus, I., Carvalheiro, L.G., Henry, M., Isaacs, R., Klein, A.M., Kremen, C., M'gonigle, L.K., Rader, R. and Ricketts, T.H. (2015) Delivery of crop pollination services is an insufficient argument for wild pollinator conservation. Nature communications, *6*(1), 1-9

Klein, A. M., Steffan–Dewenter, I., & Tscharntke, T. (2003) Fruit set of highland coffee increases with the diversity of pollinating bees. P. R. Soc. London. B: Bio., *270*(1518), 955-961

Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C., & Tscharntke, T. (2007). Importance of pollinators in changing landscapes for world crops. Proceedings of the royal society B: biological sciences, *274*(1608), 303-313.

Koeniger N., Koeniger G. (2000) Reproductive isolation among species of the genus *Apis*. Apidologie *31*(2), 313-339

Konarska, A. (2014). Characteristics of flower nectaries of *Hedera helix* L.(Araliaceae). Acta Sci. Pol. Hortorum Cultus, *13*(1), 109-122

Köppler, K., Vorwohl, G., & Koeniger, N. (2007) Comparison of pollen spectra collected by four different subspecies of the honey bee *Apis mellifera*. Apidologie, *38*(4), 341-353.

Kuhlmann, M., (2013) *Colletes hederae* The IUCN Red List of Threatened Species: e.T13306742A13309507 http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T13306742A13309507.en date accessed 21 December 2018

Kuppler, J., Wieland, J., Junker, R. R., & Ayasse, M. (2021). Drought-induced reduction in flower size and abundance correlates with reduced flower visits by bumble bees. AoB PLANTS., *13*(1), plab001.

Kutschbach-Brohl L, Washburn B E, Bernhardt G E, Chipman R B, & Francoeur L C. (2010) Arthropods of a semi-natural grassland in an urban environment: the John F. Kennedy International Airport, New York. Journal of Insect Conservation, *14*(4), 347-358

Lande, R. (1993) Risks of population extinction from demographic and environmental stochasticity and random catastrophes. The American Naturalist, *142*(6), 911-927

Larson, B. M. H., Kevan, P. G., & Inouye, D. W. (2001) Flies and flowers: taxonomic diversity of anthophiles and pollinators. The Canadian Entomologist, *133*(4), 439-465.

Larsson M., & Franzén M. (2008) Estimating the population size of specialised solitary bees. Ecological Entomology., *33*(2), 232-238

Lawson, D. A., & Rands, S. A. (2019). The effects of rainfall on plant–pollinator interactions. Arthropod-Plant Interactions, *13*(4), 561-569.

Leather S. R. (2017). "Ecological Armageddon"-more evidence for the drastic decline in insect numbers. Annals of Applied Biology, *172*(1), 1-3

Lehrer, M., & Srinivasan, M. V. (1992). Freely flying bees discriminate between stationary and moving objects: performance and possible mechanisms. Journal of Comparative Physiology *A*, *171*(4), 457-467.

Lehrer M (1998) Looking all around: honeybees use different cues in different eye regions. J Exp Biol 201:3275–3292

Liechti, F. (2006). Birds: blowin'by the wind? Journal of Ornithology, *147*(2), 202-211

López-Uribe M M, Morreale S J, Santiago C K, & Danforth B. N. (2015) Nest suitability, fine-scale population structure and male-mediated dispersal of a solitary ground nesting bee in an urban landscape. PLoS One, 10.1371/journal.pone.0125719

Macadam C R, & Bairner S Z. (2012) Urban biodiversity: Successes and challenges: Brownfields: Oases of urban biodiversity. The Glasgow Naturalist, *25*(4).

Mallinger, R. E., & Prasifka, J. R. (2017). Bee visitation rates to cultivated sunflowers increase with the amount and accessibility of nectar sugars. Journal of applied entomology, *141*(7), 561-573.

Mason, N. A. (2017). Effects of wind, ambient temperature and sun position on damselfly flight activity and perch orientation. *Animal Behaviour*, *124*, 175-181.

McAllister, H. A. (1990). *Hedera helix L.* and *H. hibernica* (Kirchner) bean (Araliaceae) in the British Isles

McKnight, D. T., & Ligon, D. B. (2017) Correcting for unequal catchability in sex ratio and population size estimates. PloS one, 12, e0184101.

McNeilage, A., Robbins, M. M., Gray, M., Olupot, W., Babaasa, D., Bitariho, R., ... & Baker, J. (2006) Census of the mountain gorilla *Gorilla beringei beringei* population in Bwindi Impenetrable National Park, Uganda. Oryx, *40*(4), 419-427

Meeus, I., Brown, M. J., De Graaf, D. C., & Smagghe, G. U. Y. (2011) Effects of invasive parasites on bumble bee declines. Conservation Biology., *25*(4), 662-671

Messing, R.H., Klungness, L.M. and Jang, E.B. (1997) Effects of wind on movement of *Diachasmimorpha longicaudata*, a parasitoid of tephritid fruit flies, in a laboratory flight tunnel. Entomologia Experimentalis et Applicata, *82*(2), 147-152

Metcalfe, D. J. (2005). *Hedera helix L*. *Journal of Ecology*, *93*(3), 632-648.

MET office, *How we measure wind*, https://www.metoffice.gov.uk/weather/guides/observations-guide/how-we-measure-wind date accessed 15 March 2019

Minckley, R. L., Wcislo, W. T., Yanega, D., & Buchmann, S. L. (1994) Behavior and phenology of a specialist bee (*Dieunomia*) and sunflower (*Helianthus*) pollen availability. Ecology, *75*(5), 1406-1419.

Minckley, R. L., & Roulston, T. H. (2006) Incidental mutualisms and pollen specialization among bees. *Plant-pollinator interactions: from specialization to generalization* (ed. By Waser N.W. & Ollteron J.), pp. 69-98, Chicago, USA.

Minckley, R. L., Roulston, T. A. H., & Williams, N. M. (2013). Resource assurance predicts specialist and generalist bee activity in drought. Proceedings of the Royal Society B: Biological Sciences, *280*(1759), 20122703.

Mirza, M. M. Q. (2003). Climate change and extreme weather events: can developing countries adapt?. *Climate policy*, *3*(3), 233-248

Møller, A.P. (2013) Long-term trends in wind speed, insect abundance and ecology of an insectivorous bird. Ecosphere, *4*(1), 1-11

Montgomery G A, Dunn R R, Fox R, Jongejans E, Leather S R, Saunders M E, Shorthall C R, Tingley M W, & Wagner D L. (2020) Is the insect apocalypse upon us? How to find out. Biological Conservation., *241*, 108327.

Müller A., & Kuhlmann M. (2008) Pollen hosts of western palaearctic bees of the genus *Colletes* (Hymenoptera: Colletidae): the Asteraceae paradox. Biological Journal of the Linnean Society *95*(4), 719-733

Murray, T. E., Kuhlmann, M., & Potts, S. G. (2009). Conservation ecology of bees: populations, species and communities. *Apidologie*, *40*(3), 211-236.

Nardone, E., Dey, T., & Kevan, P. G. (2013). The effect of sugar solution type, sugar concentration and viscosity on the imbibition and energy intake rate of bumblebees. *Journal of insect physiology*, *59*(9), 919-933.

National Research Council (2007) Status of Pollinators in North America. Washington, DC: The National Academies Press. https://doi.org/10.17226/11761.

Natural England (2011) *Habitat of the month: June 2011 Lowland meadows.* http://webarchive.nationalarchives. gov.uk/20130903135107/http://www. naturalengland.org.uk/ourwork/conservation/ biodiversity/englands/habitatofthemonth/ lowlandmeadows.aspx. Date accessed 1 January 2020

NBN atlas, *Eucera longicornis,* occurrence download at https://species.nbnatlas.org/species/NHMSYS0000875930 date accessed 1 March 2021

Nguyen, Q. V., Chan, W. L., & Debiasi, M. (2016). Hybrid design and performance tests of a hovering insect-inspired flapping-wing micro aerial vehicle. *Journal of Bionic Engineering*, *13*(2), 235-248.

Nicolson, S. W. (2011) Bee food: the chemistry and nutritional value of nectar, pollen and mixtures of the two. African Zoology, *46*(2), 197-204.

Nieto, A., Roberts, S.P.M., Kemp, J., Rasmont, P., Kuhlmann, M., García Criado, M., Biesmeijer, J.C., Bogusch, P., Dathe, H.H., De la Rúa, P., De Meulemeester, T., Dehon, M., Dewulf, A., Ortiz-Sánchez, F.J., Lhomme, P., Pauly, A., Potts, S.G., Praz, C., Quaranta, M., Radchenko, V.G., Scheuchl, E., Smit, J., Straka, J., Terzo, M., Tomozii, B., Window, J. and Michez, D. (2014) European Red List of bees. Luxembourg: Publication Office of the European Union.

Norgate, M., Boyd-Gerny, S., Simonov, V., Rosa, M. G., Heard, T. A., & Dyer, A. G. (2010). Ambient temperature influences Australian native stingless bee (*Trigona carbonaria*) preference for warm nectar. PLoS One, *5*, e12000.

Norris, M. J. (1936) The feeding-habits of the adult Lepidoptera Heteroneura. Transactions of the Royal Entomological Society of London, *85*(2), 61-90.

Nouvian, M., Reinhard, J. and Giurfa, M. (2016) The defensive response of the honeybee *Apis mellifera*. Journal of Experimental Biology, *219*(22), 3505-3517

Ogilvie, J. E., & Forrest, J. R. (2017) Interactions between bee foraging and floral resource phenology shape bee populations and communities. Current opinion in insect science, *21*, 75-82.

Ollerton, J., Winfree, R., & Tarrant, S. (2011) How many flowering plants are pollinated by animals?. Oikos, *120*(3), 321-326

Ollerton J., Erenler H., Edwards M., & Crockett R. (2014) Extinctions of aculeate pollinators in Britain and the role of large-scale agricultural changes. Science, *346*(6215), 1360-1362

Orrock, J.L. and Danielson, B.J. (2009) Temperature and cloud cover, but not predator urine, affect winter foraging of mice. Ethology, *115*(7), 641-648

Otis, G. W. (1982). Weights of worker honeybees in swarms. Journal of Apicultural Research, *21*(2), 88-92.

Owens N. (2011) Male *Anthophora plumipies.* https://www.bwars.com/bee/apidae/anthophora-plumipes date accessed 12 December 2019.

Papiorek, S., Junker, R. R., & Lunau, K. (2014). Gloss, colour and grip: multifunctional epidermal cell shapes in bee-and bird-pollinated flowers. *Plos one*, *9*, e112013.

Pasek, J.E. (1988). Influence of wind and windbreaks on local dispersal of insects. In Windbreak Technology (pp. 539-554).

Pattrick, J. G. (2018). *Grip, slip, petals, and pollinators: Linking the biomechanics, behaviour and ecology of interactions between bees and plants* (Doctoral dissertation, University of Cambridge).

Peakall, R., & Schiestl, F. P. (2004) A mark-recapture study of male *Colletes cunicularius* bees: implications for pollination by sexual deception. Behavioural Ecology and Sociobiology, *56*(6), 579-584

Peat, J., & Goulson, D. (2005). Effects of experience and weather on foraging rate and pollen versus nectar collection in the bumblebee, *Bombus terrestris*. Behavioral Ecology and Sociobiology, *58*(2), 152-156.

Pekkarinen A. (1997) Oligolectic bee species in northern Europe (Hymenoptera, Apoidea). Entomology Fennica, *8*(4), 205-214

Peterson, J. H., Roitberg, B. D., & Peterson, J. H. (2006) Impacts of flight distance on sex ratio and resource allocation to offspring in the leafcutter bee, *Megachile rotundata.* Behavioral Ecology and Sociobiology, *59*(9), 589-596

Phillips, B. B., Wallace, C., Roberts, B. R., Whitehouse, A. T., Gaston, K. J., Bullock, J. M., ... & Osborne, J. L. (2020). Enhancing road verges to aid pollinator conservation: a review. *Biological Conservation*, 108687

Pinzauti, M. (1986) The influence of the wind on nectar secretion from the melon and on the flight of bees: the use of an artificial wind-break. Apidologie, *17*(1), 63-72

Porter, S. D., & Tschinkel, W. R. (1987). Foraging in *Solenopsis invicta* (Hymenoptera: Formicidae): effects of weather and season. *Environmental Entomology*, *16*(3), 802-808.

Potts S., & Willmer P. A. T. (1997) Abiotic and biotic factors influencing nest-site selection by *Halictus rubicundus*, a ground-nesting halictine bee. Ecological Entomology, 2*2*(3), 319-328

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O., & Kunin, W. E. (2010) Global pollinator declines: trends, impacts and drivers. Trends in Ecology and Evolution, *25*(6), 345-353

Powney G. D., Carvell C., Edwards M., Morris R. K., Roy H. E., Woodcock B. A., & Isaac N. J. (2019) Widespread losses of pollinating insects in Britain. Nature Comms, *10*(1), 1-6

Purvis, A., Gittleman, J. L., Cowlishaw, G., & Mace, G. M. (2000) Predicting extinction risk in declining species. Proceedings of the Royal Society of London. B: Biological Sciences, *267*(1456), 1947-1952

Pyke, G.H. (1978) Optimal foraging: movement patterns of bumblebees between inflorescences. Theoretical population biology, *13*(1), 72-98

Pyke, G.H. (1979) Optimal foraging in bumblebees: rule of movement between flowers within inflorescences. Animal Behaviour, *27*(4), 1167-1181.

Pyke, G. H. (2016). Floral nectar: pollinator attraction or manipulation? Trends in ecology & evolution, *31*(5), 339-341.

Pywell, R. F., Webb, N. R., & Putwain, P. D. (1994) Soil fertility and its implications for the restoration of heathland on farmland in southern Britain. Biological Conservation, *70*(2), 169-181

R Core Team (2017) R: A language and environment for statistical computing. R

Rader, R., Reilly, J., Bartomeus, I., & Winfree, R. (2013). Native bees buffer the negative impact of climate warming on honey bee pollination of watermelon crops. Global change biology, *19*(10), 3103-3110.

Radford, A.N., McCleery, R.H., Woodburn, R.J.W. and Morecroft, M.D. (2001) Activity patterns of parent Great Tits *Parus major* feeding their young during rainfall. Bird Study, *48*(2), 214-220

Rasmont P, Franzén M, Lecocq T, Harpke A, …& Gonseth Y. (2015) Climatic risk and distribution atlas of European bumblebees. Pensoft Publishers *10*,1-236.

Ravi, S., Crall, J., Fisher, A. and Combes, S. (2013) Rolling with the flow: bumblebees flying in unsteady wakes. Journal of Experimental Biology, *216*(22), 4299-4309

Reinhold, K. (1999) Energetically costly behaviour and the evolution of resting metabolic rate in insects. Functional Ecology, *13*(2), 217-224

Ren, G., Liu, J., Wan, J., Li, F., Guo, Y. and Yu, D. (2018) The analysis of turbulence intensity based on wind speed data in onshore wind farms. Renewable Energy, *123*, 756-766.

Ribbands, C. R. (1949) The foraging method of individual honeybees. Journal of Animal Ecology. *18*(1), 47-66.

Ricketts, T. H., Daily, G. C., Ehrlich, P. R., & Michener, C. D. (2004) Economic value of tropical forest to coffee production. Proceedings of the National Academy of Sciences, *101*(34), 12579-12582

Riessberger, U. and Crailsheim, K. (1997) Short-term effect of different weather conditions upon the behaviour of forager and nurse honey bees (*Apis mellifera carnica* Pollmann). Apidologie, *28*(6), 411-426

Ritchie A, D,, Ruppel R,, & Jha S, (2016) Generalist behaviour describes pollen foraging for perceived oligolectic and polylectic bees. Environmental Entomology, *45*(4), 909-919

Roberts, S. & Vereecken N. (2010): *Information Sheet Ivy Bee* (*Colletes hederae*). http://www.bwars.com/sites/www.bwars.com/files/info_sheets/01_Colletes_hederae_ 20100908.pdf date accessed 12 December 2018

Roberts, S., Potts, S., Biesmeijer, K., Kuhlmann, M., Kunin, W., & Ohlemüller, R. (2011). Assessing continental-scale risks for generalist and specialist pollinating bee species under climate change. BioRisk, *6*, 1-18

Robinson E, Cipullo M., Sousounis P., Kafali C., Latchman S., Higgs S., Maisey P., Mitchell L. (2017) UK Windstorms and Climate Change. AIR worldwide & Met Office.

Rollin, O., Bretagnolle, V., Decourtye, A., Aptel, J., Michel, N., Vaissière, B. E., & Henry, M. (2013) Differences of floral resource use between honey bees and wild bees in an intensive farming system. Agriculture, Ecosystems and Environment, *179*,78-86.

Rosenheim, J. A., Nonacs, P., & Mangel, M. (1996). Sex ratios and multifaceted parental investment. The American Naturalist, *148*(3), 501-535.

Roulston, T. A. H., & Cane, J. H. (2000). The effect of diet breadth and nesting ecology on body size variation in bees (Apiformes). Journal of the Kansas Entomological Society, *73*(3),129-142.

Ruedenauer, F. A., Spaethe, J., & Leonhardt, S. D. (2016). Hungry for quality—individual bumblebees forage flexibly to collect high-quality pollen. Behavioral Ecology and Sociobiology, *70*(8), 1209-1217

Rundlöf, M., Andersson, G. K., Bommarco, R., Fries, I., Hederström, V., Herbertsson, L., ... & Smith, H. G. (2015). Seed coating with a neonicotinoid insecticide negatively affects wild bees. Nature, *521*(7550), 77-80.

Samuelson, A. E., Gill, R. J., Brown, M. J., & Leadbeater, E. (2018). Lower bumblebee colony reproductive success in agricultural compared with urban environments. *Proceedings of the Royal Society B*, *285*, 20180807.

Sánchez-Bayo, F., Goulson, D., Pennacchio, F., Nazzi, F., Goka, K., & Desneux, N. (2016). Are bee diseases linked to pesticides? A brief review. Environment international, *89*, 7-11.

Sánchez-Bayo, F., & Wyckhuys, K. A. (2019). Worldwide decline of the entomofauna: A review of its drivers. Biological conservation, *232*, 8-27

Sardiñas HS, Kremen C (2014) Evaluating nesting microhabitat for ground-nesting bees using emergence traps. Basic Appl Ecol *15*(2),161–168

Saunders P (2014) *Eucera longicornis*, The long horned mining bee in Cornwall.

Saunders, P (2017) *The long horned bee in Cornwall* http://kernowecology.co.uk/Publications/Long%20horn%20bee%20ps%202017.pdf date accessed 01 January 2020.

Saul, A. (1987). Estimation of survival rates and population size from mark-recapture experiments of bait-caught haematophagous insects. *Bulletin of entomological research*, *77*(4), 589-602.

Sawyer R., & Pickard R. S. (1981) Pollen identification for beekeepers. University College Cardiff Press.

Schmidt, J.O. (1990) Hymenopteran venoms: striving toward the ultimate defence against vertebrates. Insect defences: adaptive mechanisms and strategies of prey and predators, SUNY press, Albany, New York. pp.387-419.

Schäffler, I., & Dötterl, S. (2011) A day in the life of an oil bee: phenology, nesting, and foraging behavior. Apidologie, *42*(3), 409-424

Schmidt, K., & Westrich, P. (1993) *Colletes hederae* n. sp., eine bisher unerkannte, auf Efeu (*Hedera*) spezialisierte Bienenart (Hymenoptera: Apoidea). *Entomologische Zeitschrift*, *103*(6), 89-112.

Schoonhoven, L. M., Van Loon, B., van Loon, J. J., & Dicke, M. (2005). *Insect-plant biology*. Oxford University Press on Demand.

Schultz, C. B., & Dlugosch, K. M. (1999) Nectar and hostplant scarcity limit populations of an endangered Oregon butterfly. Oecologia, 119(2), 231-238

Schwarz, C. J., & Arnason, A. N. (1996) A general methodology for the analysis of capture-recapture experiments in open populations. Biometrics, *52*(3), 860-873.

Schultz, C. B., & Hammond, P. C. (2003). Using population viability analysis to develop recovery criteria for endangered insects: case study of the Fender's blue butterfly. *Conservation Biology*, *17*(5), 1372-1385.

Scriven L. A., Sweet M. J., & Port G. R. (2013) Flower density is more important than habitat type for increasing flower visiting insect diversity. International Journal of Ecology https://doi.org/10.1155/2013/237457

Seeley, T.D. (1989) Social foraging in honey bees: how nectar foragers assess their colony's nutritional status. Behavioral Ecology and Sociobiology, *24*(3), 181-199

Seeley, T. D. (2009). *The wisdom of the hive: the social physiology of honey bee colonies*. Harvard University Press.

Senapathi, D., Biesmeijer, J. C., Breeze, T. D., Kleijn, D., Potts, S. G., & Carvalheiro, L. G. (2015a). Pollinator conservation—the difference between managing for pollination services and preserving pollinator diversity. *Current Opinion in Insect Science*, *12*, 93-101.

Senapathi D, Carvalheiro L G, Biesmeijer J C, Dodson C A, … Potts S.G. (2015b) The impact of over 80 years of land cover changes on bee and wasp pollinator communities in England. Proceedings of the Royal Society B: Biological Sciences., *282*(1806), 20150294

Shaffer, M. L. (1981) Minimum population sizes for species conservation. BioScience, *31*(2), 131-134

Singh, H. S., & Gibson, L. (2011) A conservation success story in the otherwise dire megafauna extinction crisis: the Asiatic lion (*Panthera leo persica*) of Gir forest. Biological Conservation., *144(*5), 1753-1757

Sirohi MH, Jackson J, Edwards M, Ollerton J (2015) Diversity and abundance of solitary and primitively eusocial bees in an urban centre: a case study from Northampton (England). J Insect Conserv *19*(3),487–500

Sirois-Delisle, C., & Kerr, J. T. (2018). Climate change-driven range losses among bumblebee species are poised to accelerate. *Scientific reports*, *8*(1), 1-10.

Sommeijer, M. J., Rooijakkers, E. F., Jacobusse, C., & Kerkvliet, J. D. (2009). Larval food composition and food plants of the solitary bee Colletes halophilus (Hymenoptera: Colletidae). Journal of apicultural research, *48*(3), 149-155.

Soroye, P., Newbold, T., & Kerr, J. (2020). Climate change contributes to widespread declines among bumble bees across continents. Science, *367*(6478), 685-688.

Soulsbury, C. D., Iossa, G., Baker, P. J., & Harris, S. (2008). Environmental variation at the onset of independent foraging affects full-grown body mass in the red fox. Proceedings of the Royal Society B: Biological Sciences, *275*(1649), 2411-2418.

Statista. (2019). *Quarterly wind speed average in the United Kingdom (UK) from 2010 to 2018 (in knots)* https://www.statista.com/statistics/322789/quarterly-wind-speed-average-in-the-united-kingdom-uk/ date accessed 12 February 2019.

Steinert M., Sydenham M. A. K., Eldegard K., & Moe S. R. (2020) Conservation of solitary bees in power-line clearings: Sustained increase in habitat quality through woody debris removal. Global Ecology and Conservation., https://doi.org/10.1016/j.gecco.2019.e00823

Stelzer, R. J., & Chittka, L. (2010). Bumblebee foraging rhythms under the midnight sun measured with radiofrequency identification. BMC biology, *8*(1), 1-7.

Stephens, D.W. and Krebs, J.R. (1986) Foraging theory. Princeton University Press, Princeton NJ.

Stout, J. C., & Goulson, D. (2002). The influence of nectar secretion rates on the responses of bumblebees (*Bombus* spp.) to previously visited flowers. Behavioral Ecology and Sociobiology, *52*(3), 239-246.

Sun, J. F., Gong, Y. B., Renner, S. S., & Huang, S. Q. (2008). Multifunctional bracts in the dove tree *Davidia involucrata* (Nyssaceae: Cornales): rain protection and pollinator attraction. The American Naturalist, *171*(1), 119-124.

Sussex Wildlife Trust Information about the Seven Sisters and Seaford Head https://assets.sussexwildlifetrust.org.uk/Files/information-about-the-seven-sisters-and-seaford-head.pdf date accessed 19 December 2019

Sussex Wildlife trust. Seaford Head Nature Reserve. https://assets.sussexwildlifetrust.org.uk/swtseafordheadwalkleafletwebonly.pdf date accessed 8 January 2020

Svirejeva-Hopkins A., Schellnhuber H. J., & Pomaz V. L. (2004) Urbanised territories as a specific component of the Global Carbon Cycle. Ecological Modelling, 173(2-3), 295-312

Taylor, I. R. (1983). Effect of wind on the foraging behaviour of Common and Sandwich Terns. Ornis Scandinavica, 90-96.

Teglhøj, P. G. (2017). A comparative study of insect abundance and reproductive success of Barn Swallows *Hirundo rustica* in two urban habitats. Journal of Avian Biology, *48*(6), 846-853.

Teppner, H., & Brosch, U. (2015). Pseudo-oligolecty in *Colletes hederae* (Apidae-Colletinae, Hymenoptera). *Linzer biol. Beitr*, *47*(1), 301-306.

Tew, N. E., Memmott, J., Vaughan, I. P., Bird, S., Stone, G. N., Potts, S. G., & Baldock, K. C. (2021). Quantifying nectar production by flowering plants in urban and rural landscapes. *Journal of Ecology*. *109*(4), 17471757

The Guardian (2015) *Stinging indictment: poll reveals public ignorance about bees in the UK*. https://www.theguardian.com/environment/2015/aug/18/stinging-indictment-poll-reveals-public-ignorance-about-bees-in-the-uk date accessed 16 December 2020

Thomas C. D., Jones T. H., & Hartley S. E. (2019) “Insectageddon”: A call for more robust data and rigorous analyses. Global Changes in Biology, *25*,1891-1892.

Thomas, C. D. (2000) Dispersal and extinction in fragmented landscapes. Proceedings of the Royal Society of London. Series B: Biological Sciences., *267*(1439), 139-145

Thomas, J. A. (1995) The ecology and conservation of *Maculinea arion* and other European species of large blue butterfly. In Ecological Conservation of butterflies 180-197.

Thomas, J. A., Telfer, M. G., Roy, D. B., Preston, C. D., Greenwood, J. J. D., Asher, J., & Lawton, J. H. (2004) Comparative losses of British butterflies, birds, and plants and the global extinction crisis. Science, *303*(5665), 1879-1881

Hothorn T., Bretz F. and Westfall P. (2008) Simultaneous Inference in General Parametric Models. Biometrical Journal *50*(3), 346-363

Traill, L. W., Bradshaw, C. J., & Brook, B. W. (2007) Minimum viable population size: a meta-analysis of 30 years of published estimates. Biological Conservation *139*(1-2), 159-166

Tucker, V.A. and Schmidt-Koenig, K. (1971) Flight speeds of birds in relation to energetics and wind directions. The Auk, *88*(1), 97-107

Tuell, J.K. and Isaacs, R. (2010) Weather during bloom affects pollination and yield of highbush blueberry. Journal of economic entomology, *103*(3), 557-562

Upham P., Thomas C., Gillingwater D., & Raper D. (2003) Environmental capacity and airport operations: current issues and future prospects. Journal of Air Transport Management, *9*(3), 145-151

Vance, J. T., Faruque, I., & Humbert, J. S. (2013). Kinematic strategies for mitigating gust perturbations in insects. Bioinspiration & biomimetics, *8*, 016004.

Vanderplanck, M., Vereecken, N. J., Grumiau, L., Esposito, F., Lognay, G., Wattiez, R., & Michez, D. (2017) The importance of pollen chemistry in evolutionary host shifts of bees. Scientific reports, *7*(1), 1-10.

Van Dyck H., Van Strien A. J., Maes D., & Van Swaay C. A. (2009) Declines in common, widespread butterflies in a landscape under intense human use. Conservation Biology, *23*(4), 957-965

Vaudo, A. D., Tooker, J. F., Grozinger, C. M., & Patch, H. M. (2015). Bee nutrition and floral resource restoration. Current opinion in insect science, *10*, 133-141.

Vicens, N. and Bosch, J. (2000) Weather-dependent pollinator activity in an apple orchard, with special reference to *Osmia cornuta* and *Apis mellifera* (Hymenoptera: Megachilidae and Apidae). Environmental Entomology, *29*(3), 413-420

Vogt, F. D. (1986). Thermoregulation in bumblebee colonies. I. Thermoregulatory versus brood-maintenance behaviors during acute changes in ambient temperature. Physiological zoology, *59*(1), 55-59.

Voigt, C. C., Schneeberger, K., Voigt-Heucke, S. L., & Lewanzik, D. (2011). Rain increases the energy cost of bat flight. Biology letters, *7*(5), 793-795.

Waddington, K. D. (1990). Foraging profits and thoracic temperature of honey bees (*Apis mellifera*). Journal of Comparative Physiology B, *160*(3), 325-329.

Wagner, D. L., Metzler, K. J., & Frye, H. (2019). Importance of transmission line corridors for conservation of native bees and other wildlife. Biological conservation, *235*, 147-156.

Wakefield, E.D., Phillips, R.A., Matthiopoulos, J., Fukuda, A., Higuchi, H., Marshall, G.J. and Trathan, P.N. (2009) Wind field and sex constrain the flight speeds of central-place foraging albatrosses. Ecological Monographs, *79*(4), 663-679

Walters, K. F. A., & Dixon, A. F. G. (1984). The effect of temperature and wind on the flight activity of cereal aphids. Annals of Applied Biology, *104*(1), 17-26.

Warren M. S., Hill J. K., Thomas J. A., Asher J., Fox, R…. Jeffcoate G. (2001) Rapid responses of British butterflies to opposing forces of climate and habitat change. Nature, *414*(6859), 65-69

Warren, J. and James, P. (2008) Do flowers wave to attract pollinators? A case study with Silene maritima. Journal of Evolutionary Biology, *21*(4), 1024-1029

Warren, J. (2015). Is wind-mediated passive leaf movement an effective form of herbivore defence?. Plant Ecology and Evolution, *148*(1), 52-56.

Wasielewski, O., Wojciechowicz, T., Giejdasz, K., & Krishnan, N. (2013). Overwintering strategies in the red mason solitary bee—physiological correlates of midgut metabolic

activity and turnover of nutrient reserves in females of Osmia bicornis. *Apidologie*, *44*(6), 642-656.

Wcislo W. T., & Cane J. H. (1996) Floral resource utilization by solitary bees (Hymenoptera: Apoidea) and exploitation of their stored foods by natural enemies. Annual Review of Entomology, *41*(1), 257-286

Weimerskirch, H., Guionnet, T., Martin, J.S.S.A., Shaffer, S.A. and Costa, D.P. (2000) Fast and fuel efficient? Optimal use of wind by flying albatrosses. Proceedings of the Royal Society of London B: Biological Sciences, *267*(1455), 1869-1874

Weimerskirch, H., Bonadonna, F., Bailleul, F., Mabille, G., Dell'Omo, G. and Lipp, H.P. (2002) GPS tracking of foraging albatrosses. Science, *295*(5558), 1259

Westrich P. (1996) Habitat requirements of central European bees and the problems of partial habitats. In Linnean Society Symposium. Series. Vol. *18*,1-16.

Westrich, P. (2008) Flexibles Pollensammelverhalten der ansonsten streng oligolektischen Seidenbiene *Colletes hederae* Schmidt & Westrich (Hymenoptera: Apidae). *Eucera*, *1*(2), 17-29.

Whitaker, D.M., Carroll, A.L. and Montevecchi, W.A. (2000) Elevated numbers of flying insects and insectivorous birds in riparian buffer strips. Canadian journal of zoology, *78*(5), 740-747

Whitney, H., Federle, W., & Glover, B. (2009). Grip and slip: mechanical interactions between insects and the epidermis of flowers and flower stalks. Communicative & integrative biology, *2*(6), 505-508.

Whitney, H. M., & Federle, W. (2013). Biomechanics of plant–insect interactions. Current opinion in plant biology, *16*(1), 105-111.

Wignall, V. R., Alton, K., & Ratnieks, F. L. (2019). Garden centre customer attitudes to pollinators and pollinator-friendly planting. PeerJ, *7*, e7088.

Wignall, V. R., Brolly, M., Uthoff, C., Norton, K. E., Chipperfield, H. M., Balfour, N. J., & Ratnieks, F. L. (2020). Exploitative competition and displacement mediated by eusocial bees: experimental evidence in a wild pollinator community. Behavioral Ecology and Sociobiology, *74*(12),1-15.

Wiklund C., & Fagerström T. (1977) Why do males emerge before females? Oecologia, *31*(2), 153-158

Williams, I. H. (1994). The dependence of crop production within the European Union on pollination by honey bees. Agric. Zool. Rev., *6*, 229-257.

Williams, C. S. (1998) The identity of the previous visitor influencesflower rejection by nectar-collecting bees. Animal Behaviour, *56*(3), 673-681

Williams, N. M., & Kremen, C. (2007). Resource distributions among habitats determine solitary bee offspring production in a mosaic landscape. *Ecological applications, 17*(3), 910-921.

Willmer, P. G., Bataw, A. A. M., & Hughes, J. P. (1994). The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology*, *19*(3), 271-284.

Willmer, P. (2012). Ecology: pollinator–plant synchrony tested by climate change. Current Biology, *22*(4), 131-132.

Winfree R., Bartomeus I., & Cariveau D. P. (2011) Native pollinators in anthropogenic habitats. Annual Review of Ecology, Evolution and Systematics, *42*,1-22.

Winfree, R., Aguilar, R., Vázquez, D. P., LeBuhn, G., & Aizen, M. A. (2009) A meta-analysis of bees' responses to anthropogenic disturbance. Ecology, *90*(8), 2068-2076

Wolf, E., & Zerrahn-Wolf, G. (1937). Flicker and the reactions of bees to flowers. The Journal of general physiology, *20*(4), 511-518.

Wolf, T. J., Ellington, C. P., & Begley, I. S. (1999). Foraging costs in bumblebees: field conditions cause large individual differences. Insectes sociaux, *46*(3), 291-295.

Wood T. J., Holland J. M., & Goulson D. (2017) Providing foraging resources for solitary bees on farmland: current schemes for pollinators benefit a limited suite of species. Journal of Applied Ecology, *54*(1) 323-333

Wood, T. J., Kaplan, I., & Szendrei, Z. (2018) Wild bee pollen diets reveal patterns of seasonal foraging resources for honey bees. Frontiers in Ecology and Evolution, *6*,210.

Wood, T. J., Michez, D., Paxton, R. J., Drossart, M., Neumann, P., Gérard, M., ... & Vereecken, N. J. (2020). Managed honey bees as a radar for wild bee decline?. *Apidologie*, 1-17.

Woodcock, B. A., Bullock, J. M., Shore, R. F., Heard, M. S., Pereira, M. G., Redhead, J., ... & Peyton, J. (2017) Country-specific effects of neonicotinoid pesticides on honey bees and wild bees. Science, *356*(6345), 1393-1395

Wright S (1933) Inbreeding and homozygosis. Proc Natl Acad Sci USA *19*(4),411–420

Wuellner C. T. (1999) Nest site preference and success in a gregarious, ground-nesting bee *Dieunomia triangulifera.* Ecological Entomology, *24*(4).

Yamazaki, K. (2011). Gone with the wind: trembling leaves may deter herbivory. Biological Journal of the Linnean Society, *104*, 738-747, 471-479

Yanega D. (1990) Philopatry and nest founding in a primitively social bee, *Halictus rubicundus.* Behavioural Ecology and Sociobiology., *27*(1), 37-42

Zanette L. R. S., Martins R. P., Ribeiro S. P. (2005) Effects of urbanization on Neotropical wasp and bee assemblages in a Brazilian metropolis. Landscape and Urban Planning *71*(2-4), 105-121

Zattara, E. E., & Aizen, M. A. (2021). Worldwide occurrence records suggest a global decline in bee species richness. One Earth, *4*(1), 114-123.

Zayed A. (2004) Effective population size in Hymenoptera with complementary sex determination. Heredity, *93*(6), 627-630

Zayed, A., & Packer, L. (2007) The population genetics of a solitary oligolectic sweat bee, *Lasioglossum (Sphecodogastra) oenotherae* (Hymenoptera: Halictidae). Heredity, *99*(4), 397-405

Zayed, A. (2009) Bee genetics and conservation. Apidologie, *40*(3), 327-262

Zeng, Z., Ziegler, A. D., Searchinger, T., Yang, L., Chen, A., Ju, K., ... & Wood, E. F. (2019). A reversal in global terrestrial stilling and its implications for wind energy production. Nature Climate Change, *9*(12), 979-985.

Zhang, S. W., Xiang, W., Zili, L., & Srinivasan, M. V. (1990). Visual tracking of moving targets by freely flying honeybees. Visual neuroscience, *4*(4), 379-386.

Zurbuchen A., Landert L., Klaiber J., Müller A., Hein S., & Dorn S. (2010a) Maximum foraging ranges in solitary bees: only few individuals have the capability to cover long foraging distances. Biological Conservation. *143*(3), 669-676

Zurbuchen A., Cheesman S., Klaiber J., Müller A., Hein S., & Dorn S. (2010b) Long foraging distances impose high costs on offspring production in solitary bees. Journal of Animal Ecology, *79*(3), 674-781

Zuur, A. F., Ieno, E. N., & Elphick, C. S. (2010). A protocol for data exploration to avoid common statistical problems. Methods in ecology and evolution, *1*(1), 3-14.

Appendix A- Stinging risk and sting pain of the Ivy bee, Colletes hederae

A1.1 Abstract

The ivy bee, *Colletes hederae* was first detected in Britain in 2001 and is now abundant in many locations. Ivy bee ground nesting aggregations can occur in public areas and cause concern about stinging risk. Here we assess that risk. The likelihood of being stung or collided with when in an active aggregation was low, with only 1 sting in 10 hours of activity (standing, walking, "gardening") by a human subject. Collisions were low at 6 per hour. The ivy bee sting is frequently unable to penetrate human skin. Only half were able to sting the fingertips and 75% the inner forearm when a bee was held against these areas. Pain was scored using a modified Starr/Schmidt scale. Ivy bee stings were significantly less painful

than those of honey bee workers *Apis mellifera*, with similar scores to a nettle sting, *Urtica dioica*. Pain subsided after 10 minutes with weals smaller than both honey bees and nettles. The ivy bee venom sac was c. 39% the volume of that of a worker a honey bee. The sting is similar in size to the worker honey bee sting and barbs could not be seen at 35X magnification. We conclude that the ivy bee is not a danger to the public. They rarely sting people active in a nest aggregation area, have a sting that is frequently unable to penetrate human skin and that causes minimal pain for a short duration, comparable to that of a nettle sting. Nettle stings are generally considered to be annoying and unpleasant, but not dangerous.

A1.2 Introduction

Female Aculeata, bees, wasps and ants in the order Hymenoptera, possess a sting (Branstetter et al. 2017), although in some groups such as the Formicinae ants (Blum & Hermann 1978) and Meliponini bees (Kerr & Lello 1962, Shackleton et al 2015) the sting has been evolutionarily lost. Males do not have a sting, as it is part of the female reproductive system (Van Marle & Piek 1986). The stings of female Hymenoptera vary widely in the pain they cause to humans. Starr (1985) and Schmidt et al (1984) categorized sting pain on a scale from 0 to 4. The sting of the honey bee, *Apis mellifera*, is given a score of 2, or "painful". The honey bee is a common insect and, from personal experience, many people know that its sting can cause considerable pain. Similarly, yellowjacket wasps and European hornets score 2. A score of 4 is "traumatically painful", with three species noted: the bullet ant Paraponera clavata, the social wasp Synoeca septentrionalis and the tarantula hawk wasp Pepsis formosa. A score of zero is "no penetration" and a score of 1 is "so slight as to constitute no real deterrent". The ranking of sting pain has been continued by Schmidt, who developed the well-known Schmidt Sting Pain Index (1990, 2016).

The ivy bee, *Colletes hederae*, is a ground nesting solitary bee that was only identified as a distinct species in 1993 from individuals caught in Germany and Croatia (Bischoff et al 2005). It recently colonized Britain from continental Europe with the first record in 2001 from Langton Matravers, Dorset (Roberts & Vereecken 2010), a few kilometres from the south coast. An ongoing mapping project by the UK interest group BWARS (Bees, Wasps & Ants Recording Society) showed that by 2017 there were many records from the southern third of Britain, with some as far north as the Yorkshire and Lancashire coasts, 400 km from the first record (BWARS 2018).

The ivy bee can be very common at British sites and in other European countries (Kuhlmann 2018) with individuals forming large nesting aggregations both in Britain and continental European populations (Bischoff et al 2005, Falk & Lewington 2015, BWARS 2018; Fig 1a, Fig1e; see Results). In the UK, ivy bees are on the wing from August to November, but most abundant in September and early October (personal observations). Ivy bees can frequently be seen foraging on ivy flowers alongside honey bees and many other insects (Fig 1a-c) (Garbuzov & Ratnieks 2014), where they probably go unrecognized by the general public as a distinct species. However, their aggregations of ground nests can be noticeable and even alarming due to the large numbers of bees seen "swarming" above the nests. These include

females leaving and returning from foraging trips and also many males flying just above ground level and on the ground searching and grappling for mates. The nests, with entrance holes and small piles of excavated soil, can also be conspicuous (Fig 1f). Although there are many species of ground-nesting bees in Britain (Falk & Lewington 2015), having large numbers of ground nesting bees active in autumn is a new phenomenon. This has resulted in members of the public becoming concerned for their safety and that of their children and contacting pest control officers of local councils and local grounds keepers (personal communication from Mr. Joseph Arnold, Animal Control Officer, Lewes District Council and East Sussex and Horstead Garden Services, East Sussex) sometimes with demands for control or elimination. The ivy bee is approximately the same size as the honey bee, and quite similar in colour. This can also result in the neighbours of beekeepers commenting that some of the beekeeper's bees have moved into their garden, and would they like them returned (FR, personal observation).

Is the sting of the ivy bee a danger to the public? The BWARS website notes that although female ivy bees can sting they do not sting people and that "*C. hederae* is all but completely safe with children and pets". Given that there is some public concern, here we systematically asses the danger. First, we quantified the rate at which a person present in an active nest aggregation is stung or bumped into by ivy bees. Second, we determined the ability of the ivy bee sting to penetrate human skin. Third we quantified the pain caused by the sting and the duration and size of the weal or swelling caused and how these compared to stings of worker honey bees and stinging nettles (*Urtica dioica*). Stinging nettles are common in the UK. Most people have first-hand experience of being stung by them. As the nettle sting is considered more annoying than painful and is not a safety issue, it is a useful benchmark for an innocuous sting whereas the honey bee representing a painful sting. In addition, we compared the size of the sting and venom sac of ivy and honey bees as a step in identifying any morphological factors that may influence sting penetration and pain.

A1.3 Methods

A.3.1 Aggression within nest aggregations

Two nest aggregations were studied in detail. Aggregation 1 was found in a garden in the village of Falmer, Sussex, close to the University of Sussex campus (Fig 1e). Aggregation 2 was smaller and on the University of Sussex campus (US Arts). Due to its larger size and

higher density of nests (Table 1), more time was allocated to aggregation 1 in determining the potential danger of being stung by ivy bees.

In aggregation 1, three 3 x 3m squares with nests at densities typical for the aggregation were studied. In the smaller aggregation 2, two 3 x 3m squares were studied. Within each square the number of nests was determined by counting the entrance holes/soil excavation piles. A square was then allocated to either "standing" or "walking" for 20 minute periods. In aggregation 1 a third activity, "gardening" was also made. Standing consisted of an observer standing still in one square but moving to a new part every 5 minutes. Walking was continuous around a square at a normal speed. For gardening the observer was on their knees, patting the grass and mimicking certain gardening behaviours. As ivy bee aggregations can occur in flower beds, lawns, and beside foot paths (Fig1e -f), these three activities were intended to mimic what people could be doing within them.

Every five minutes the number of flying bees was estimated by swiping an insect net twice across the whole square at close to ground level, where the bees were most abundant. The captured bees were then counted, sexed and released. Throughout each 20 minute period the number of times a bee collided with the head or body of the observer, or if the observer was stung, were recorded. The number of collisions and stings were also measured with the observer standing just outside the aggregation, 1-2 meters from the last nest on that side of the aggregation. Here data were collected in 30 minute periods for a total of two hours. Data were collected in good weather, when bee flight activity was occurring (temperatures> 15°C, sunny, little or no wind,10:30-16:00, 27 September to 15 October 2018).

A1.3.2 Sting penetration: fingertip and inner forearm

Data on sting penetration were collected on 9 October, 2015. Female ivy bees foraging on ivy flowers on the University of Sussex campus and in the neighbouring village of Falmer were captured. Each of the three observers (FR, KS & NB) then held, for approximately 20 seconds, one bee between thumb and index finger in such a way that the bee could flex its abdomen to sting, or attempt to sting, a fingertip. We observed whether the bee everted her sting and if the sting was able to penetrate the flesh. The bee, still held between thumb and finger, was then placed in contact with the skin of the inner forearm, which is generally softer but less pain sensitive than the fingers (Smith 2014), for 20 seconds, and the sting test repeated. Due to the lack of aggression from ivy bees, stings had to be induced by directly holding an individual against the skin.

A1.3.3 Sting effect: pain level and weal size

In 2018 the pain of ivy bee, honey bee and nettle stings were scored using a modified Starr/Schmidt scale (Starr 1985, Schmidt 1990); 2: the pain of a typical honey bee sting; 1.5: less painful but still definitely painful; 1: borderline between painful and an irritable or mildly unpleasant sensation; 0.5: can be felt as irritating, but definitely not painful; 0.25: borderline between able to feel something versus nothing; 0: no sensation. Although given numbers, these pain levels are categorical, not numerical. The size and duration of any weals caused were also measured. Pairs (n=7) of ivy bee and honey bee stings were administered to a single subject (FR) by a second person (GH) with one sting on each inner forearm within 2 minutes.

The order of sting presentation was randomised. The subject was blind to the species of bee that was stinging each forearm for the initial observation (0 minutes). The subject however was not blind at later observations because the effects of honey bee and ivy bee sings are very different. Bees were collected immediately before (<5 minutes) stinging at nest entrances from a nearby aggregation or from the entrance of a hive at the laboratory. This ensured that only older honey bees with a full venom reservoir were used (Nouvian et al 2016).

To administer an ivy bee sting, the bee was held between thumb and index finger and the abdomen pressed gently against the subject's forearm for 20seconds. This generally resulted in the bee stinging, sometimes making several closely spaced venom injections. In a few cases a bee did not sting, so a second bee was used. The process for administering honey bee stings was similar. When a honey bee stung the subject, the detached sting apparatus was left in the arm for 20 seconds before being scraped away. The subject's reaction (pain, and weal size) was recorded at intervals (0, 2 ,5 ,10, 30, 60 minutes and 3, 6, 12, 24 hours). The weal was defined as the area surrounding the sting site where there was swelling or reddening of the skin or both. The length and width of this area was measured to 1mm using a ruler. One pair of stings was administered per day, except for one occasion where one round was administered in the morning and another three hours later. In this case distinct areas of the forearm were used.

Nettle stings were administered to two subjects (GH & FR) from stinging nettle plants, *Urtica dioica*, found locally. This allowed comparison between pain from ivy bee stings to a very common sting that almost everyone in the UK experiences because nettle plants are very widespread and abundant. Nettle stings are generally considered to be non-harmful and both

adults and children are often stung. A small piece of stem or leaf with stinging hairs was cut from a freshly picked nettle stem. This was rubbed on the forearm of the subject in such a way as to ensure only a single sting was administered, although in one trial two stings occurred. In this case, only one of the weals was used for measurements. Stings were applied to both forearms on three days per subject. Pain and weal size were recorded as with the bee stings. The area of the weal was not always circular, so was approximated to an ellipse (length/2*width/2)*π.

All subjects used to investigate sting penetration and pain gave written consent to be used in the study. Due to all subjects being authors of the paper ethical approval was not sought.

A1.3.4 Sting size: comparing ivy bee and honey bee

To quantify sting length and venom sac size females were collected from nest or hive entrances and dissected on the collection day. Each bee was pinned under water, ventral side up for honey bees and dorsal side up for ivy bees, to assist removing the sting and observing the venom sac *in situ* in the ivy bee. The following morphological measurements were made: sting length, base of sting width, middle of sting width, length and width of the venom sac. The volume of the venom sac was calculated as $4/3\pi$ x (length/2) x $(\text{width}/2)^2$ as venom sacs were roughly spheroid in shape. We used a Euromex Steroblue dissection microscope fitted with an eye piece reticule (sensitvity±0.007mm).

To compare body sizes, female ivy bees and worker honey bees foraging on ivy were captured, placed in a vial and weighed in the field using a GEM20 smart weight scale, sensitivity 0.001g.

A1.3.5 Statistics

To determine if the number of collisions per bee (collisions divided by the total number of bees caught per five-minute sweep) was significantly different between activities, a general linear model with a Tweedie distribution was used to control for zero inflation of the data. To compare sting lengths a t-test was used. To compare the width of the base of the stinger and venom sac volume Mann-Whitney U tests were used as the data were not normally distributed. Comparisons between ivy bee, honey bee and nettle sting pain and weal area were made using Mann-Whitney U tests at 0, 10, 30 and 60 minutes and 3, 12 and 24 hours after stinging. Bee weights were compared using a t-test. All statistical tests were completed in R 3.5.1 (R core team 2017).

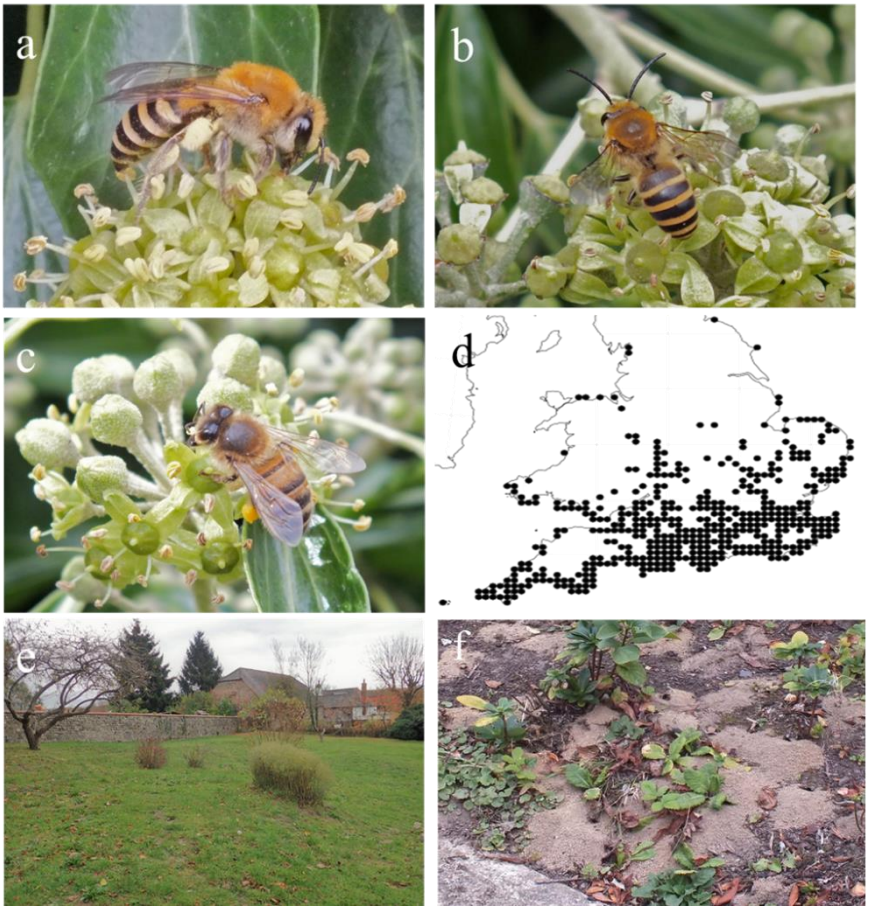


Fig 1: a) female ivy bee, b) male ivy bee, c) honey bee, all foraging on ivy, d) BWARS 2017 distribution map of the ivy bee in Britain, e) Aggregation 1,Falmer, the largest of the aggregations studied and in the lawn of a village house, f) noticeable excavation produced by nesting ivy bees in a flower bed

A1.4 Results

A1.4.1 Description of aggregations

Aggregation 1 was 350m^2 with an estimated 4000 nests, c. 12 per m^2. Aggregation 2 was 64m^2 with an estimated 120 nests at 2 nests per m^2. Ivy bee activity was highest at aggregation 1, with an average of 19 bees per swiping, versus only 3 at aggregation 2 (Table 1).

Table 1: Estimated numbers of nests for the 3 study aggregations on or near to the University of Sussex campus (US). Also shown are total bees caught over the entire study period for the two main aggregations and the percentage of females and males. The mean number is from the 5 swipe nettings (n=155) made every 5 minutes during all observer activities at the aggregation.

Aggregation	Size (m^2)	Estimated number of nests	Total females (%)	Total males (%)	Mean number females per swiping	Mean number males per swiping
1-Falmer	350	4,150	2330 (85%)	400 (15%)	16.1	2.8
2-US Arts	64	115	167 (97%)	5 (3%)	3.3	0.1
3-US Lab	21	43	-	-	-	-

A1.4.2 Risk of being stung whilst in an aggregation when bees were active

The observer was only stung once while carrying out the walking activity, across all 3 activities for 10 hours in total. Figure 2 shows that the number of collisions was low, with an average across activities of 0.02 per bee, or 6 bees per hour across both aggregations combined and 8 per hour in aggregation 1, the larger aggregation. There was no significant difference between the different activities and how often a collision occurred per bee (General linear model, $F_{(2,152)}=2.73$, $P=0.07$; Fig 2). No collisions occurred when the observer stood 1m outside aggregation 1 for a total of 2 hours.

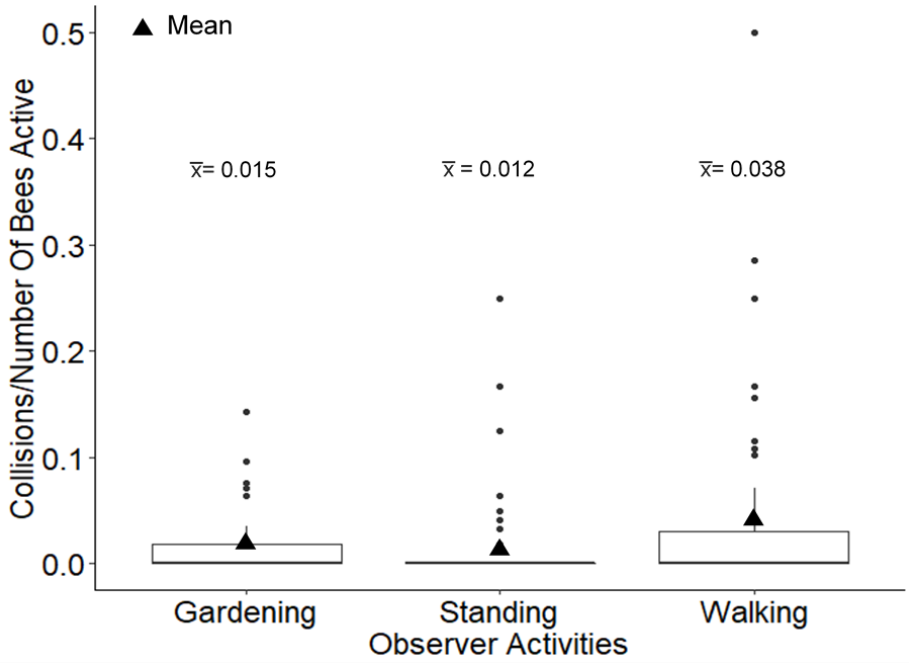


Fig 2: Number of collisions with human observer in the aggregation per number of bees active (calculated as number of collisions divided by the total number of bees caught in the swipe nettings). Data combined for both collisions to observer head and body across both aggregations (Falmer and US Arts) are shown (number of 5 minute observation periods=155)

A1.4.3 Sting penetration

In the 2015 tests, almost all ivy bees held between thumb and finger everted their sting (31/32, 97%), but only half were able to penetrate the fingertip skin (16/31, 52%) and sting. There were differences among the 3 observers in the number of bees able to penetrate (Observer FR 1/12; KS 7/10; NB 8/10). Fisher's Exact Tests with Bonferroni correction shows that FR was stung less often than either KS ($p = 0.0186$) or NB ($p = 0.0045$), but that KS and NB were stung equally ($p = 1$). For stings to the forearm, observer FR was again stung less (6/10), but not significantly less than either KS (9/10) or NB (8/10) (total 23/30, 77%, Fisher's exact test with Bonferroni correction $p=0.430$).

A1.4.4 Sting pain

Direct comparisons of honey bee and ivy bee sting pain indicate that the initial pain level of a honey bee sting is significantly higher than an ivy bee (Mann Whitney U, $p= 0.001$,

d_{cohen}=2.87). All honey bee stings scored 2 on the modified Schmidt/Starr pain scale compared to 1 or less for ivy bees (Fig 3a-b). Honey bee stings were significantly more painful than ivy bee stings for up to three hours (Mann Whitney U, 10 minutes p=0.004, d_{cohen}= 1.96, 30 minutes p=0.001, d_{cohen}=2.87, 3 hours p=0.001, d_{cohen}=2.87) but not for longer durations (Mann Whitney U, 12 hours p=0.48, d_{cohen}= 0.03, 24 hours p=0.35, d_{cohen}=0.20).

Nettle sting pain and weal size were not significantly different between the two subjects (t. test, $t_{(98)}$=1.25, p=0.215, d_{cohen}=0.263, $t_{(98)}$=0.172, p=0.864, d_{cohen}=0.394) and so were combined. There was no difference in pain score for ivy bee and nettle stings at any time point (Fig3c, Mann Whitney U, 0 minutes p=0.448, d_{cohen}=0.130, 10 minutes p=0.433, d_{cohen}=0.078, 30 minutes p=0.248, d_{cohen}=0.316 3, 12 and 24 hours p=0.484, dcohen=0.212) with all stings of both species being scored 0 at 12 and 24 hours.

Weal size was significantly larger for the honey bee compared to the ivy bee for the first 12 hours (Mann Whitney U 10 minutes p=0.001 d_{cohen}=1.25, 30, 60 minutes and 3 hours p= 0.001, d_{cohen}=2.87 and 12 hours p=0.03, d_{cohen}=1.13). Nettle stings also caused larger weals than ivy bee stings for the first 3 hours (Mann Whitney U 10 minutes p=0.023, d_{cohen}=1.026, 30 minutes p= 0.005, d_{cohen}=1.43, 3 hours p=0.011, d_{cohen}=1.23, 12 hours p= 0.075, d_{cohen}=0.70). Ivy bee stings caused the least amount of red, on average, across all time periods (2.73mm^2) followed by stinging nettle (31.2mm^2) with both much less than honey bee (1053.6mm^2). For honey bee stings, weal area was greatest three hours after stinging (1638mm^2). Weals reached an average

maximum of 11.72mm^2 for the ivy bee 10 minutes after stinging. The maximum average for the common nettle was at 30 minutes, 96.4mm^2.

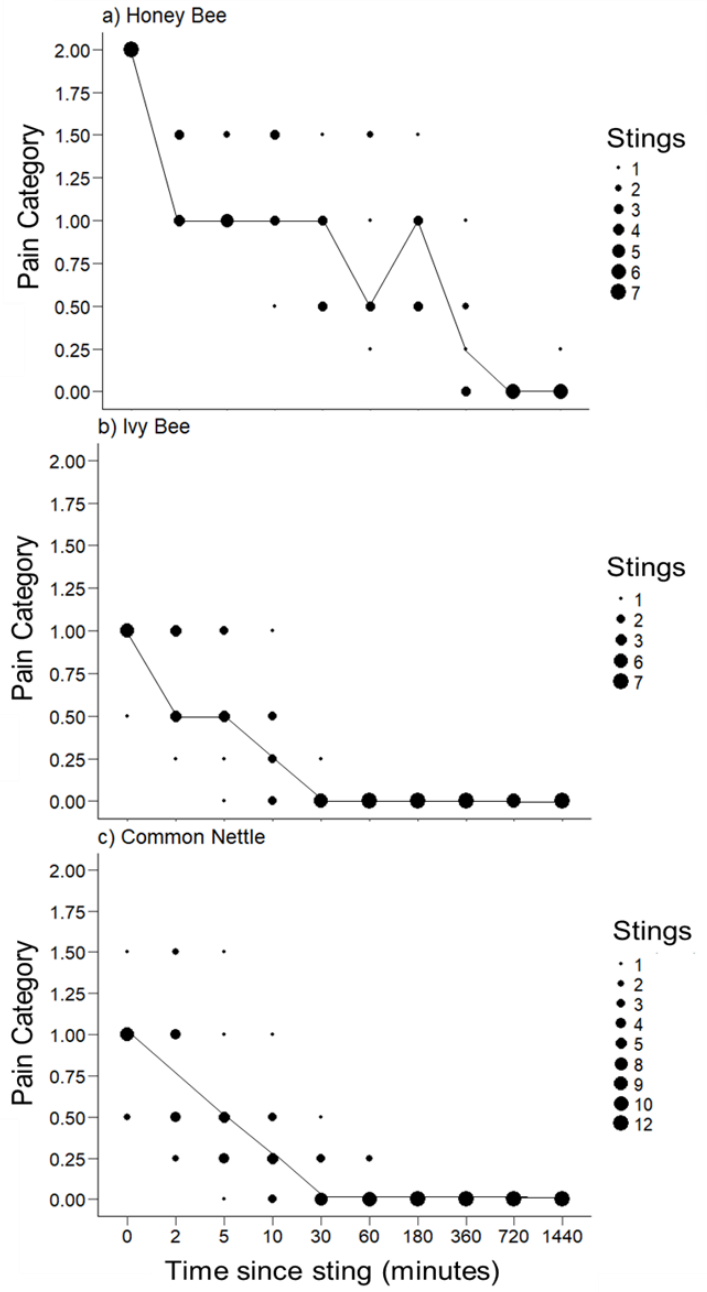


Fig 3: Sting pain duration starting with the initial sting and continuing for 24 hours. Scores were made using a modified Schmidt/Starr pain scale and are categorical (categories are 2, 1.5, 1, 0.5, 0.25, 0) Lines display median pain rank through time. Size of circle relates to number of stings. Significances are reported in text.

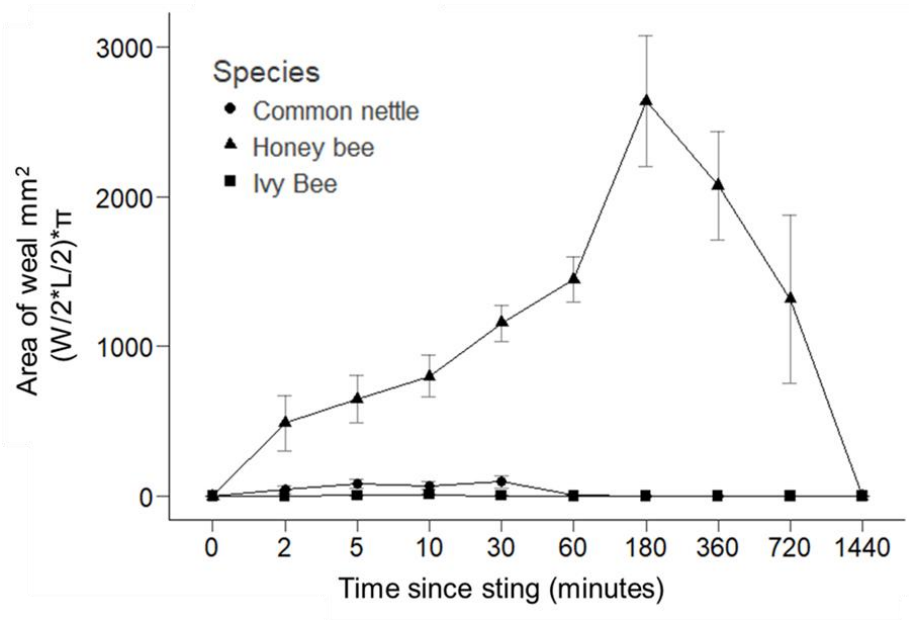


Fig 4: Weal area caused by a sting for the three study species. Standard error bars are shown. Significances are reported in text

A1.4.5 Comparison of sting morphology

A total of 10 honey bees and 14 ivy bees were dissected. Ivy bee stingers were significantly longer (mean=2.45± 0.08 mm) than those of worker honey bees (mean=2.06 0±0.04 mm) (t. test, $t_{(22)}$=-3.97, p=0.0006, d_{cohen}=1.74, Fig 5a) but narrower at the base (Wilcoxon signed rank, W=79.5, p=0.002, d_{cohen}=2.99). There was no difference in the middle width of stings between the two species (t. test, $t_{(22)}$= -1.63, p=0.12, d_{cohen}=0.47). Honey bee stings have easily visible barbs at the tip (Fig6c), whereas any barbs present on the ivy bee sting were not visible at 35x magnification. The ivy bee sting is curved (Fig6d). Honey bee venom sacs were 89% larger than ivy bees (honey bee $\bar{x}$= 0.886mm^3±0.112, ivy bee $\bar{x}$=0.342mm^3±0.156), a significant difference (Wilcoxon signed rank, W=80, p=0.003, d_{cohen}=1.31, Fig 5c). When dissecting out honey bee venom sacs they would easily leave the body along with the sting due to autotomy (Fig6a). Ivy bee venom sacs did not (Fig6b) and when removed were easily

broken. Foraging ivy bee females were significantly heavier than foraging worker honey bees ($\bar{x}$ =116.7 ±3.37mg versus $\bar{x}$=87.8 ± 2.32mg, t test, t_{23}=-6.33, p<0.0001 d_{cohen}=2.72).

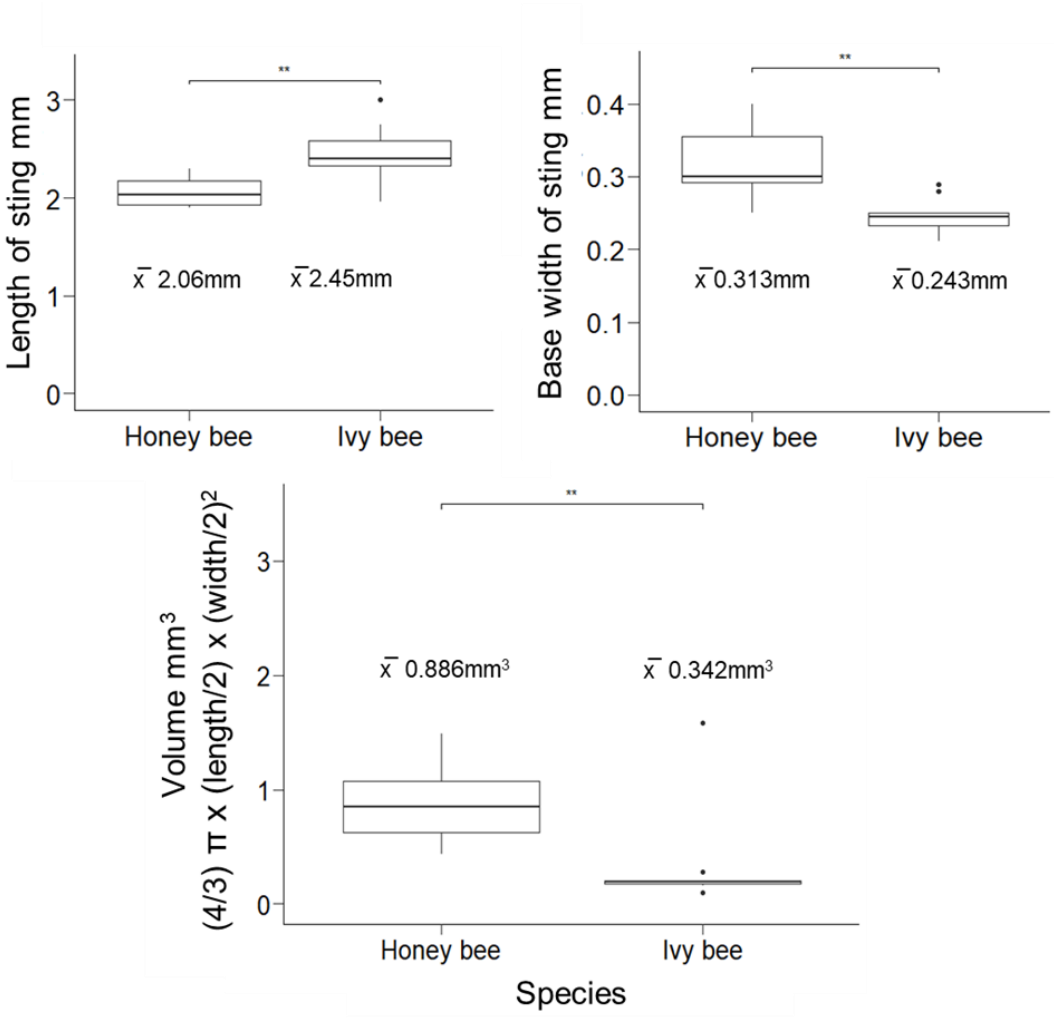


Fig 5: Morphometric parameters of the ivy bee and honey bee stings; a) length of sting from base to tip, b) width of base of sting, c) volume of venom sac. Whiskers indicate 1.5x IQR

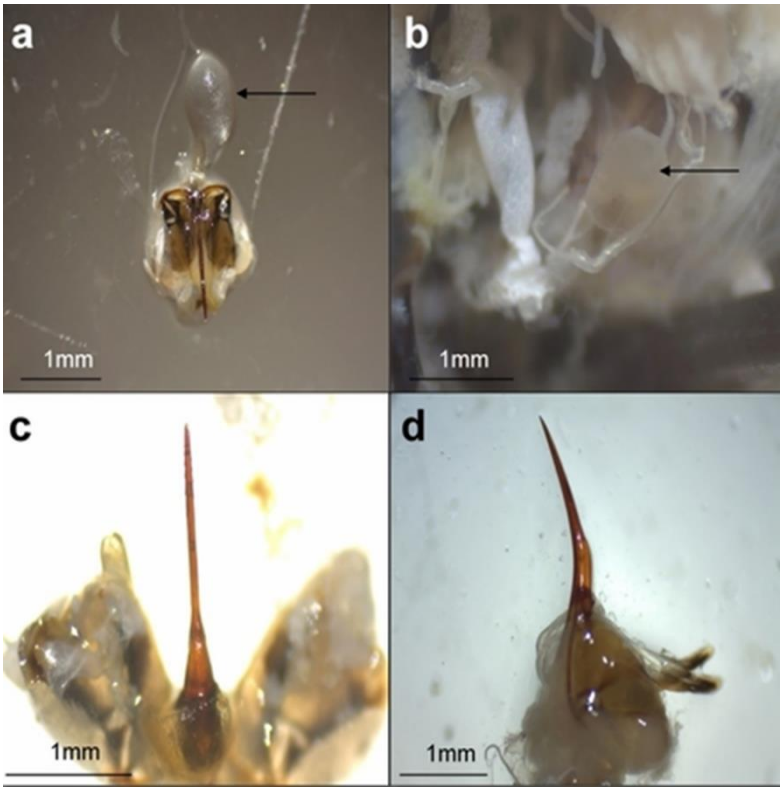


Fig 6: Images of the stinging apparatus for honey bee workers and ivy bee. a) the honey bee venom sac attached to the sting, b) the venom sac for the ivy bee, still located inside the body, c) the barbed honey bee sting and d) the longer and more curved sting of the ivy bee

A1.5. Discussion

Our results clearly show that the ivy bee poses little risk to the public. Being stung is rare and the sting will often not penetrate human skin. If it does, the pain is minimal, much less than that of a honey bee sting and similar to that of a stinging nettle. The weal produced is also small, even slightly smaller than from a nettle sting.

When a person carried out activities within an ivy bee aggregation, collisions with flying bees were infrequent. Even in the largest study aggregation, with an estimated 4000 nests, only eight collisions occurred per hour. These collisions appeared to be accidental, not aggressive, in contrast to the actively aggressive defensive behaviours exhibited by honey bees near their nests or hives. When defending their colony honey bees are well known for flying towards an intruder, often buzzing around the head and purposefully making physical contact (Collins and Kubasec 1982). Activity type (walking, standing, gardening) did not significantly affect the likelihood of a collision, indicating that increasing observer activity

levels (e.g. standing<walking) does not affect ivy bee defensive behaviour, nor does it appear to increase incidental collision rate.

Being stung was even less frequent, with the observer receiving only one sting in the 10 hours spent within the aggregations. Again, this is much less than for honey bees, with one study showing that within 30 seconds of a synthetic alarm pheromone being released adjacent to a hive, honey bees had stung the intruder a total of 10 times (Collins et al 1982).

Even when clearly attempting to sting, by curving the abdomen and everting the stinger, ivy bee stings have a limited ability to penetrate human skin. Most tested bees, 97%, attempted to sting when held between finger and thumb but only 51% of attempts were successful in penetrating fingertip skin. When stinging the inner forearm individual ivy bees were more successful, with 75% of these resulting in a sting, perhaps due to the skin being thinner and therefore easier to penetrate (Fruhstorfer et al 2000, Sandby-Moller et al 2003). Honey bee workers would sting immediately and were always successful. When ivy bees were placed against a forearm they took longer to deliver a sting than honey bees. Often, they would miss due to having to curl the body, potentially to increase the penetration power (Herman 1971, Hermann & Gonzalez 1986).

When directly comparing ivy bee to honey bee stings, ivy bee stings were always ranked as significantly less painful, even though the subject (FR) has been stung 10-20 thousand times by honey bee during bee keeping activities but had never been stung by an ivy bee prior to this investigation. As such, the subject was almost certainly less sensitive to honey bee stings than non-beekeepers, which reinforces the fact that he reported honey bee stings as more painful. The pain from an ivy bee sting always lasted less than 10 minutes and only resulted in a small weal. Honey bee stings resulted in weals almost 400 times larger in area when averaged over the 24 hour time period (Fig 4, Fig S1). The pain caused by an ivy bee sting was very similar to that of a stinging nettle (Fig 3a) but resulted in a weal approximately 12 times smaller (Fig 4). As stinging nettles are not considered to be a danger to the public, the same attitude should probably be adopted for ivy bees.

How does the ivy bee sting compare to other solitary bees? Schmidt 2016 tested and ranked the pain of eight solitary bee species, with most ranking 0.5-1.5, except for two *Xylocopa* bee species, which are as large as bumblebees, ranking 2 and 2.5 The ivy bee, with its initial sting pain never being ranked above 1 (Fig3b), follows the trend of solitary bee species stings generally being less painful than honey bees. This suggests that the honey bee sting is more

painful because it is used in nest deference against large enemies including vertebrates. Solitary bee nests are probably not often consumed by vertebrate predators, which are the main target for defensive invertebrate stings, (Herman 1971, Schmidt 2014) as they lack large food reserves.

The lack of requirement for an effective defensive sting may also be a contributing factor in the morphological differences between ivy bee and honey bee stings. Venom sac volume was only 39% of the honey bee workers, despite female ivy bees being 20% heavier. The average area of ivy bee induced weals over the 24 hours after stinging was only 0.26% of the from honey bee stings, suggesting that perhaps ivy bee venom is not as potent as honey bee venom, or that the entire contents of the venom sac are not injected. Honey bees release approximately 90% of their venom sac within 20 seconds of stinging (Schumacher et al 1994) and the entire sting apparatus becomes detached through autotomy, resulting in the bee's death. This is not the case with ivy bees, meaning they are able to sting multiple times. This ability to sting more than once may also result in less venom injected per sting, as has been reported in Vespidae wasps (Bilo 2005).

Although the pain from an ivy bee sting is low, there is still the potential for an allergic reaction, however no known allergic reaction to an ivy bee sting has been reported so far. For an allergy to manifest an individual must first be sensitised to the venom via exposure (Galli et al 2008). Our study shows that being stung once is a rare occurrence, indicating a very low risk of being stung twice. Cross over in allergic response to different insect species does occur, for example between honey bees and bumblebees (Stapel & Raadt 1998). However, these groups are more closely related phylogenetically, suggesting a greater possible overlap in venom composition and allergic cross over response (Kazuma et al 2017; Rybak-Chmielewska & Szczêsna 2004), more than would be expected with the more distantly related ivy bee (Branstetter et al 2017).

Overall our results are reassuring in terms of public safety. It would seem that the colonisation of Britain by a new bee species, which is now common and nests close to places where people live and are active, is not a danger. Ivy bees rarely sting, and any sting- induced pain subsides rapidly and is comparable to the pain of a nettle sting. It seems that ivy bees can coexist safely with humans with minimal risks to safety and that improved education and awareness of this would be beneficial. The ivy bee may look superficially like a honey bee worker, and is of similar size, but its sting is far less painful and rarely delivered.

A1.6 References

Bees, Wasps & Ants Recording Society, 2018, *Colletes hederae* [ONLINE] http://www.bwars.com/bee/colletidae/colletes-hederae. [Accessed 12 December 2018]

Bilo, B.M., Rueff, F., Mosbech, H., Bonifazi, F., Oude-Elberink, J.N.G. and EAACI Interest Group on Insect Venom Hypersensitivity, 2005. Diagnosis of Hymenoptera venom allergy. *Allergy*, **60**(11), pp.1339-1349.

Bischoff, I., Eckelt, E. and Kuhlmann, M., 2005. On the biology of the ivy-bee *Colletes hederae* Schmidt & Westrich, 1993 (Hymenoptera, Apidae). *Bonner Zoologische Beiträge*, 53, pp.27-36.

Blum, S. and Hermann, H.R., 1978. Venoms and venom apparatuses of the Formicidae: Dolichoderinae and Aneuretinae. In Arthropod Venoms (pp. 871-894).

Branstetter, M.G., Danforth, B.N., Pitts, J.P., Faircloth, B.C., Ward, P.S., Buffington, M.L., Gates, M.W., Kula, R.R. and Brady, S.G., 2017. Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Current Biology*, **27**(7), pp.1019-1025.

Charpin, D., Birnbaum, J., Lanteaume, A. and Vervloet, D., 1992. Prevalence of allergy to hymenoptera stings in different samples of the general population. *Journal of Allergy and Clinical Immunology*, **90**(3), pp.331-334.

Collins, A.M., Rinderer, T.E., Harbo, J.R. and Bolten, A.B., 1982. Colony defense by Africanized and European honey bees. *Science*, **218**(4567), pp.72-74.

Collins, A.M. and Kubasek, K.J., 1982. Field test of honey bee (Hymenoptera: Apidae) colony defensive behavior. *Annual Entomological Society of America*, **75**(4), pp.383-387.

Falk, S.J., Lewington R., 2015. Field guide to the bees of Great Britain and Ireland. British Wildlife Publishing.

Fruhstorfer, H., Abel, U., Garthe, C.D. and Knüttel, A., 2000. Thickness of the stratum corneum of the volar fingertips. *Clinical Anatomy*, **13**(6), pp.429-433.

Galli, S.J., Tsai, M. and Piliponsky, A.M., 2008. The development of allergic inflammation. *Nature*, **454**(7203), p.445.

Garbuzov, M. and Ratnieks, F.L., 2014. Ivy: an underappreciated key resource to flower-visiting insects in autumn. *Insect Conservation and Diversity*, **7**(1), pp.91-102.

Hermann, H.R., 1971. Sting autotomy, a defensive mechanism in certain social Hymenoptera. *Insectes Sociaux*, **18**(2), pp.111-120.

Hermann, H.R. and Gonzalez, J.M., 1986. Venom apparatus of Trypoxylon clavatum clavatum (Hymenoptera: Sphecidae). *Journal of Kansas Entomological Society*, pp.213-218.

Kazuma, K., Ando, K., Nihei, K.I., Wang, X., Rangel, M., Franzolin, M.R., Mori-Yasumoto, K., Sekita, S., Kadowaki, M., Satake, M. and Konno, K., 2017. Peptidomic analysis of the venom of the solitary bee *Xylocopa appendiculata circumvolans*. *Journal of Venomous Animals and Toxins*, **23**(1), p.40.

Kerr, W.E. and de Lello, E., 1962. Sting glands in stingless bees: a vestigial character (Hymenoptera: Apidae). *Journal of New York Entomological Society*, **70**(4), pp.190-214.

Kerr, J. T., Pindar, A., Galpern, P., Packer, L., Potts, S. G., Roberts, S. M., ... & Pantoja, A. (2015). Climate change impacts on bumblebees converge across continents. *Science*, *349*(6244), 177-180.

Kuhlmann, M. 2013. *Colletes hederae* The IUCN Red List of Threatened Species: e.T13306742A13309507 [ONLINE] http://dx.doi.org/10.2305/IUCN.UK.2013-2.RLTS.T13306742A13309507.en [Accessed 21 December 2018]

Mason, N. A. (2017). Effects of wind, ambient temperature and sun position on damselfly flight activity and perch orientation. *Animal Behaviour*, *124*, 175-181.

Nardone, E., Dey, T., & Kevan, P. G. (2013). The effect of sugar solution type, sugar concentration and viscosity on the imbibition and energy intake rate of bumblebees. *Journal of insect physiology*, *59*(9), 919-933.

Nouvian, M., Reinhard, J. and Giurfa, M., 2016. The defensive response of the honeybee Apis mellifera. *Journal of Experimental Biology*, **219**(22), pp.3505-3517.

Ohashi, K., & Yahara, T. (2002). Visit larger displays but probe proportionally fewer flowers: counterintuitive behaviour of nectar-collecting bumble bees achieves an ideal free distribution. *Functional Ecology*, *16*(4), 492-503.

R Core Team (2017). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/

Roberts, S. & Vereecken N. (2010): Information Sheet Ivy Bee (*Colletes hederae*). [ONLINE] http://www.bwars.com/sites/www.bwars.com/files/info_sheets/01_Colletes_hederae_20100908.pdf [Accessed 12 December 2018]

Rybak-Chmielewska, H. and Szczêsna, T., 2004. HPLC study of chemical composition of honeybee (Apis mellifera L.) venom. *Journal of Apicultural Science*, **48**(2), pp.103-9.

Sandby-Moller, J., Poulsen, T. and Wulf, H.C., 2003. Epidermal thickness at different body sites: relationship to age, gender, pigmentation, blood content, skin type and smoking habits. *ActaDV*, **83**(6), pp.410-413.

Schmidt, J. O., M. S. Blum and W. L. Overal. 1984. Hemolytic activities of stinging insect venoms. Arch. Insect Biochem. Physiol. 1: 155-60.

Schmidt, J.O., 1990. Hymenopteran venoms: striving toward the ultimate defence against vertebrates. Insect defences: adaptive mechanisms and strategies of prey and predators, SUNY press, Albany, New York. pp.387-419.

Schmidt, J.O., 2014. Evolutionary responses of solitary and social Hymenoptera to predation by primates and overwhelmingly powerful vertebrate predators. *Journal of Human Evolution*, **71**, pp.12-19.

Schmidt, J. O. 2016. "The Sting of the Wild". Baltimore, MD: Johns Hopkins Univ. Press. Pp 221-230.

Schumacher, M.J., Tveten, M.S. and Egen, N.B., 1994. Rate and quantity of delivery of venom from honeybee stings. *Journal of allergy and clinical immunology*, **93**(5), pp.831-835.
Shackleton, K., Al Toufailia, H., Balfour, N.J., Nascimento, F.S., Alves, D.A. and Ratnieks, F.L., 2015. Appetite for self-destruction: suicidal biting as a nest defense strategy in Trigona stingless bees. *Behavioral ecology and sociobiology*, **69**(2), pp.273-281.

Stapel, S.O., Waanders-Lijster de Raadt, J., Van Toorenenbergen, A.W. and De Groot, H., 1998. Allergy to bumblebee venom. II IgE cross-reactivity between bumblebee and honeybee venom.*Allergy*, **53**(8), pp.769-777.

Starr, C.K., 1985. A simple pain scale for field comparison of hymenopteran stings. *Journal of Entomological Science*, **20**(2), pp.225-232.

Van Marle J., Piek T., (1986) Venoms of the Hymenoptra Academic press, London
Book chapter: Van Marle J, Piek (1986) Morphology of the venom apparatus pp 17-44

Appendix B- Chapter two

B1: Route for the additional survey completed on 28 May in 2018. Route length was approximately 4.5km and started and ended at coordinates 50.791003, 0.156665.

B2: The number of males and females caught for each survey day in 2018 and the number of recaptures.

Date	Males Caught	Females Caught	Male Recaptures	Female Recaptures
04.05.2018	9	2	-	-
05.05.2018	10	0	3	0
08.05.2018	8	0	1	0
11.05.2018	5	3	1	0
15.05.2018	5	0	2	0
17.05.2018	4	4	3	0
20.05.2018	4	1	1	0
23.05.2018	3	4	0	0
05.06.2018	1	4	0	1
06.06.2018	1	4	0	2
11.06.2018	0	6	0	2
13.06.2018	0	12	0	0
18.06.2018	0	2	0	0
Total	**50**	**42**	**11**	**5**

B3: The number of males and females caught for each survey day in 2019 with the number of recaptures for each day. There were no female recaptures for any of the survey days in 2019.

Date	Males Caught	Females Caught	Male recaptures	Female recaptures
15.04.19	3	1	-	-
18.04.19	12	0	0	0
23.04.19	14	1	0	0
26.04.19	11	0	2	0
29.04.19	14	5	1	0
01.05.19	19	3	6	0
03.05.19	15	4	2	0
06.05.19	17	3	3	0
10.05.19	19	6	6	0
13.05.19	7	8	4	0
15.05.19	9	2	4	0
18.05.19	9	4	2	0
20.05.19	4	3	2	0
25.05.19	6	1	2	0
28.05.19	0	0	0	0
31.05.19	0	4	0	0
06.06.19	1	9	0	0
14.06.19	0	1	0	0
22.06.19	0	2	0	0
Total	**160**	**57**	**43**	**0**

B4: The models used for each year and sex to calculate population estimates are in bold alongside the alternative models not used. Due to no female recaptures in 2019 no population estimates were possible. Φ (the apparent survival rate), p (capture probability), pent (probability of entry into the population) and N (super-population size).

Year	Sex	Model	No. of parameters	AIC	Deviance
2018	**M**	**Φ{.},p{t},pent{t}**	**19**	**196.15**	**-56.66**
2018	M	Φ{t},p{.},pent{t}	26	218.94	-90.92
2018	M	Φ{t},p{t},pent{t}	36	432.62	-93.44
2018	**F**	**Φ{.}, p{t}, pent{t}**	**22**	**202.74**	**-50.85**
2018	F	Φ{.}, p{.}, pent{t}	10	109.5	-68.10
2018	F	Φ{t}, p{.}, pent{t}	9	141.02	-33.03
2019	**M**	**Φ{.}, p{.}, pent{t}**	**14**	**506.21**	**-313.5**
2019	M	Φ{t}, p{t}, pent{t}	37	511.72	-373.95
2019	M	Φ{.}, p{t}, pent{t}	31	525.10	-341.09

B5: All plant species identified in the survey. The flowering period relates to the dates when a single flower was seen on one or more transects. The average abundance was from all transects walked in the study area both for the dates when flowering and for the whole study period. Flower surveys occurred on five days.

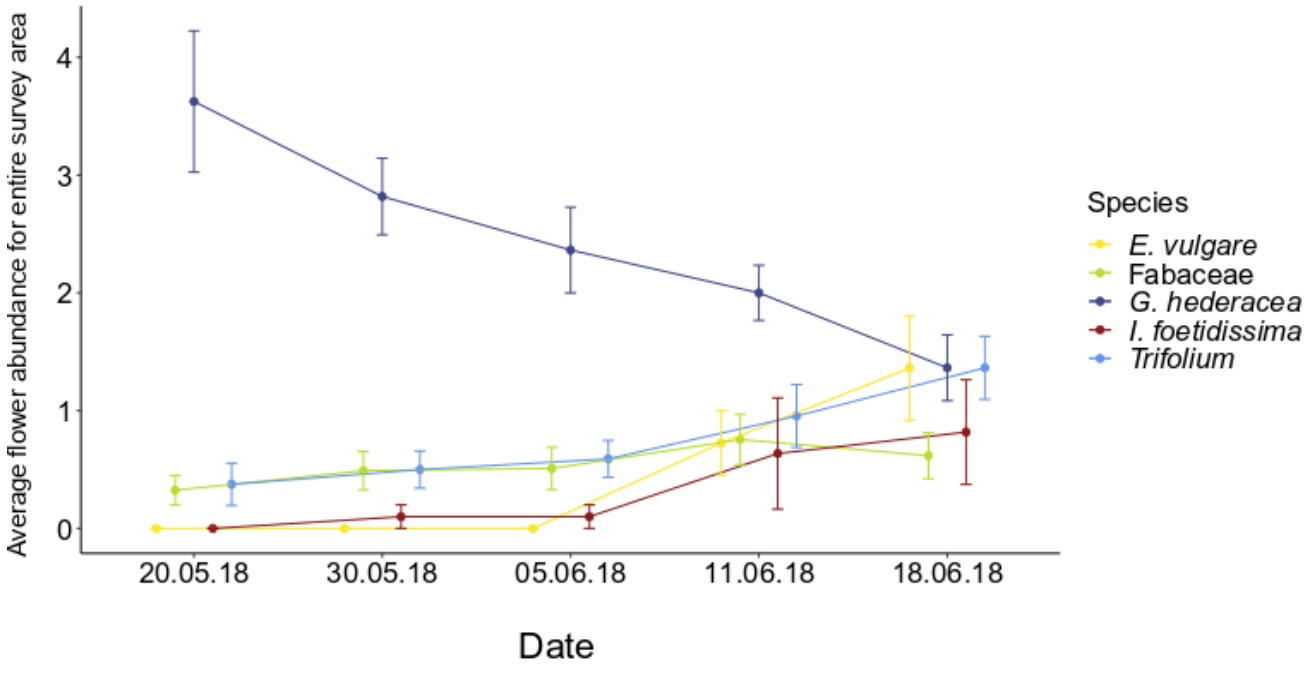
Plant Species	Flowering Dates	Average abundance throughout study period	Average abundance throughout flowering period
Agrimonia	18.06.18	0.13	0.64
Anagallis arvensis	11.06.18	0.04	0.18
Armeria maritima	30.05.18-11.06.18	0.49	0.82
Atropa belladonna	20.05.18-30.05.18	0.88	0.88
Bellis perennis	05.06.18-18.06.18	0.47	0.59
Bryonia	11.06.18-18.06.18	0.20	0.33
Carduus nutans	11.06.18	0.09	0.09
Centaurea scabiosa	11.06.18-18.06.18	0.13	0.32
Centaurium erythraea	18.06.18	0.07	0.36
Chamaenerion angustifolium	18.06.18	0.04	0.18
Cirsium arvense	11.06.18-18.06.18	0.27	0.69
Cirsium dissectum	11.06.18-18.06.18	0.24	0.59
Cirsium palustre	18.06.18	0.02	0.09
Convolvulus arvensis	11.06.18-18.06.18	0.16	0.64
Crataegus monogyna	20.05.18	0.55	2.75
Cynoglossum officinale	20.05.18-18.06.18	1.04	1.05

Daucus carota	18.06.18	0.02	0.09
Digitalis	18.06.18	0.04	0.18
Echium vulgare	11.06.18-18.06.18	0.45	1.04
Filipendula vulgaris	18.06.18	0.16	0.36
Galium mollugo	18.06.18	0.05	0.27
Geranium robertianum	18.06.18	0.04	0.18
Glaucium flavum	30.05.18-11.06.18	0.09	0.15
Glechoma hederacea	20.05.18-18.06.18	2.48	2.48
Helminthotheca echioides	20.05.18-18.06.18	0.37	0.37
Hippocrepis comosa	18.06.18	0.02	0.09
Hyacinthoides sp.	20.05.18	0.08	0.38
Hypericum perforatum	18.06.19	0.13	0.64
Iris foetidissima	30.05.18-18.06.18	0.33	0.41
Lapsana	18.06.18	0.04	0.18
Lathyrus pratensis	20.05.18-05.06.18	1.13	1.88
Leucanthemum vulgare	18.06.18	0.04	0.18
Ligustrum ovalifolium	20.05.18-18.06.18	1.99	1.99
Lonicera	11.06.18-18.06.18	0.20	0.50
Lotus corniculatus	11.06.18-18.06.18	1.27	3.18
Malva moschata	18.06.18	0.05	0.27
Medicago lupulina	18.06.18	0.09	0.45
Myosotis	20.05.18-18.06.18	0.69	0.70
Oenanthe crocata	20.05.18-11.06.18	0.66	0.82
Pentaglottis sempervirens	18.06.18	0.05	0.27
Polygala serpyllifolia	11.06.18-18.06.18	0.09	0.23
Primula veris	20.05.18	0.13	0.63
Prunella vulgaris	11.06.18-18.06.18	0.33	0.89
Ranunculus	20.05.18-18.06.18	2.51	2.51
Rosa canina	05.06.18-18.06.18	0.13	0.21
Rubus fruticosus	05.06.18-18.06.18	0.74	1.18
Silene dioica	20.05.18-18.06.18	0.70	0.70
Silene latifolia	20.05.18-05.06.18	0.16	0.26
Silene vulgaris	11.06.18-18.06.18	0.14	0.36
Solanum dulcamara	18.06.18	0.04	0.18
Stellaria media	20.05.18-18.06.18	1.10	1.10
Taraxacum	20.05.18-18.06.18	0.72	0.72
Thymus polytrichu	11.06.18-18.06.18	0.47	1.18
Trifolium pratense	20.05.18-18.06.18	0.43	0.42
Trifolium repens	20.05.18-18.06.18	1.09	1.09
Ulex europaeus	20.05.18-05.06.18	0.25	0.41
Veronica chamaedrys	20.05.18-18.06.18	1.69	1.69
Vicia cracca	11.06.18-18.06.18	0.04	0.09
Vicia sativa	20.05.18-11.06.18	0.23	0.29

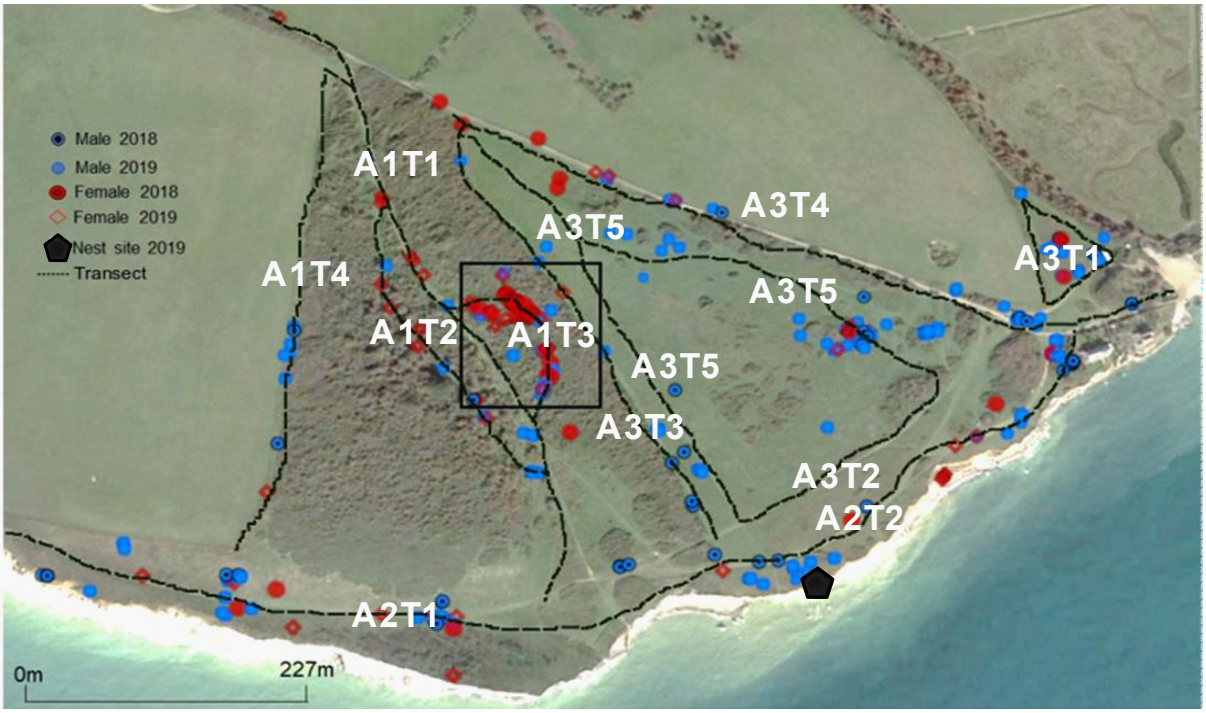
B6: The mean number of females caught and the mean abundance of the five main flower groups per transect from surveys conducted in 2018. Average abundance of flower groups was calculated only from dates when species were in flower

Transect	Mean female capture 2018	Mean female capture 2019	Mean *Glechoma hederacea* abundance (% ±SE)	Mean Fabaceae species abundance (% ±SE)	Mean *Trifolium* species abundance (% ±SE)	Mean *Iris foetidissima* abundance (% ±SE)	Mean *Echium vulgare* abundance (% ±SE)
A1T1	0.11±0.11	0.31±0.13	32 ± 20.6	22 ± 13.6	20 ± 12.6	0	8.0 ± 4.90
A1T2	0	0.46±0.14	56 ± 7.48	0	24 ± 16.0	0	0
A1T3	3.11±1.23	1.08±0.33	60 ± 10.95	0	0	44 ± 19.4	0
A1T4	0	0.08±0.08	36 ± 7.48	47 ± 8.0	18 ± 8.0	0	4 ± 4
A2T1	0.22±0.15	0.46±0.18	72 ± 10.2	77 ± 10.9	12 ± 12.0	0	28 ± 17.4
A2T2	0.22±0.15	0.23±0.12	48 ± 4.90	49.9± 16.1	12 ± 12.0	0	16 ± 7.48
A3T1	0.11±0.11	0.23±0.23	28 ± 15.0	56 ± 16.0	8.0 ± 8.0	0	4.0 ± 4.0
A3T2	0	0	48 ± 15.0	36 ± 22.3	8.0 ± 8.0	0	18 ± 9.17
A3T3	0	0.08±0.08	60 ± 8.94	62 ± 4.90	8.0 ± 8.0	0	24 ± 14.7
A3T4	0.33±0.17	0.46±0.22	24 ± 11.7	56 ± 14.70	6.0 ± 6.0	0	16 ± 11.7
A3T5	0.22±0.15	0.46±0.31	60 ± 10.95	32 ± 13.60	22 ± 5.83	0	0

B7: The average flower abundance of the 5 most visited flower species by *A. retusa* by survey date. The most visited flower species were those which either accounted for 20% of the pollen collected or 20% of the flower visits) by survey.



B8: Map of GPS locations where both male (blue) and female (red) *A. retusa* were caught in 2018 and 2019. The location of a potential nest site on the cliff edge identified in 2019 is displayed with as a black pentagon. The black rectangle indicates a 'hotspot' of female activity. Black dashed lines show the transect routes and each is labelled. A1 indicates transect is in the scrub habitat, A2 is coastal and A3 grassland.



Appendix C- Chapter three

C1: The number of males and females caught and the recapture number for Aggregation 1 per sampling day for all years. Total caught and re-caught for that year are shown. No males were caught in 2017 due to survey occurring outside of male flight period.

Year	Date	Females caught	Female recaptures	Male caught	Male recaptures
2017	13 June	5	0	-	-
	14 June	11	1	-	-
	20 June	13	3	-	-
	30 June	13	4	-	-
	1 July	11	6	-	-
	Total	**53**	**14**	**-**	**-**
2018	26 May	3	0	55	0
	1 June	3	0	43	7
	10 June	16	2	11	3
	15 June	19	0	8	4
	22 June	26	8	4	2
	28 June	27	3	0	0
	6 July	13	3	0	0
	11 July	9	3	0	0
	Total	**116**	**19**	**121**	**19**
2019	21 May	0	0	23	0
	27 May	11	0	38	9
	1 June	26	0	12	8
	9 June	31	4	5	5
	17 June	32	8	0	0
	21 June	29	8	0	0
	27 June	29	9	0	0
	1 July	23	7	0	0
	4 July	27	11	0	0
	8 July	27	18	0	0
	11 July	28	17	0	0
	Total	**263**	**82**	**78**	**22**

C2: The number of males and females caught and the recapture number for Aggregation 2 per sampling day for all years. Total caught and re-caught for that year are shown. No males were caught in 2017 due to survey occurring outside of male flight period.

Year	Date	Females caught	Female recaptures	Male caught	Male recaptures
2017	15 June	3	0	-	-
	16 June	8	0	-	-
	21 June	5	3	-	-
	2 July	5	2	-	-
	4 July	2	2	-	-
	Total	**23**	**7**	**-**	**-**
2018	25 May	2	0	17	0
	4 June	9	0	10	1
	8 June	21	3	5	3
	12 June	13	2	6	3
	21 June	17	5	5	5
	26 June	21	11	0	0
	04 July	19	9	0	0
	8 July	4	4	0	0
	Total	**106**	**34**	**43**	**12**
2019	23 May	4	0	17	0
	30 May	19	1	3	3
	5 June	12	1	1	4
	11 June	11	2	4	0
	20 June	9	3	0	0
	24 June	11	4	0	0
	28 June	13	4	0	0
	2 July	6	3	0	0
	5 July	6	2	0	0
	9 July	9	5	0	0
	13 July	7	7	0	0
	Total	**107**	**32**	**25**	**7**

C3: Models used to calculate population estimates for both aggregations and all years. Models for each sex were calculated separately and model assumptions were tested using goodness of fit. Models were chosen based on AIC values and deviance. Due to sampling later in the flight season in 2017 no male captured occurred.

Aggregation 1					
Year	Sex	Model	No. of parameters	AIC	Deviance
2017	F	Phi{.}, p{.},pent{t}	6	110.9	-71.7
2018	M	Phi{.},p{t},pent{t}	8	151.8	-192.8
	F	Phi{.},p{.},pent{t}	10	181.6	-307.2
2019	M	Phi{.},p{.},pent{t}	5	133.4	-70.8
	F	Phi{.},p{.},pent{t}	11	578.1	-628.1
Aggregation 2					
2017	F	Phi{.},p{.},pent{t}	6	64.1	-5.42
2018	M	Phi{.},p{t},pent{t}	9	122.4	-26.9
	F	Phi{.},p{.},pent{.}	7	250.3	-172.1
2019	M	Phi{.},p{.},pent{t}	6	66.5	-31.0
	F	Phi{.},p{.},pent{t}	10	271.5	-190.1

C4: **Real parameter estimates of acceptable Jolly-Seber (POPAN) models:** apparent survival (Phi), capture probability (p) and entrance probability (pent) are presented. For 2017 due to only female recaptures both aggregation 1 and 2 are presented in the same table. For 2018 and 2019 model outputs are presented separately for each aggregation showing both female and male model parameters.

1- 2017 Females

a) Aggregation 1					b) Aggregation 2				
			95% Confidence Interval					95% Confidence Interval	
Parameter	Estimate	SE	Lower	Upper	Parameter	Estimate	SE	Lower	Upper
Phi1	0.98	0.03	0.82	1.00	Phi1	0.89	0.05	0.75	0.95
p1	0.30	0.09	0.15	0.51	p1	0.55	0.21	0.18	0.87
Pent 1	0.23	0.194	0.03	0.72	Pent1	0.57	0.57	0.20	0.87
Pent2	0.25	0.20	0.04	0.73	Pent2	0	0	0	1.00
Pent3	0.16	0.18	0.01	0.73	Pent3	0.17	0.17	0.05	0.46
N	55.50	9.11	44.2	82.6	N	20.9	20.9	16.9	41.9

2- 2018 Aggregation 1

a) Females

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.91	0.03	0.83	0.96
p1	0.20	0.08	0.09	0.96
Pent 1	0.03	0.03	0	0.24
Pent2	0.24	0.08	0.12	0.43
Pent3	0.20	0.08	0.08	0.40
Pent4	0.17	0.08	0.07	0.39
Pent5	0.27	0.09	0.14	0.48
N	287.30	69.40	192.38	477.70

b) Males

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.93	0.044	0.78	0.98
p1	0.20	0.06	0.11	0.34
p2	0.10	0.07	0.02	0.33
p3	0.09	0.09	0.01	0.43
p4	0.09	0.12	0.00	0.60
Pent1	0.77	0.06	0.64	0.86
N	236.07	51.47	167.39	380.34

3- 2019 Aggregation 1

a) Females

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.94	0.00	0.92	0.96
p1	0.22	0.03	0.16	0.28
Pent1	0.24	0.07	0.13	0.39
Pent2	0.17	0.07	0.08	0.35
Pent3	0.13	0.07	0.05	0.33
Pent4	0.08	0.07	0.01	0.35
Pent5	0.10	0.06	0.03	0.30
Pent6	0.04	0.06	0.00	0.43
Pent7	0.05	0.05	0.00	0.29
Pent8	0.05	0.04	0.01	0.24
N	379.81	36.85	319.68	466.02

b) Males

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.36	0.066	0.25	0.50
p1	0.72	0.14	0.40	0.91
Pent1	0.53	0.08	0.37	0.68
Pent2	0.01	0.05	0	0.98
N	69.32	9.96	59.61	105.16

4- 2018 Aggregation 2

a) Females

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.95	0.01	0.92	0.97
p1	0.27	0.05	0.18	0.39
Pent1	0.24	0.09	0.10	0.46
Pent2	0.38	0.10	0.20	0.59
Pent3	0.20	0.10	0.07	0.47
Pent4	0.13	0.10	0.02	0.46

b) Males

Parameter	Estimate	SE	95% Confidence Interval Lower	Upper
Phi1	0.92	0.03	0.84	0.96
p1	0.21	0.08	0.00	0.39
p2	0.25	0.11	0.09	0.52
p3	0.42	0.19	0.13	0.77
p4	0.49	0.28	0.10	0.90
Pent1	0.67	0.10	0.46	0.83

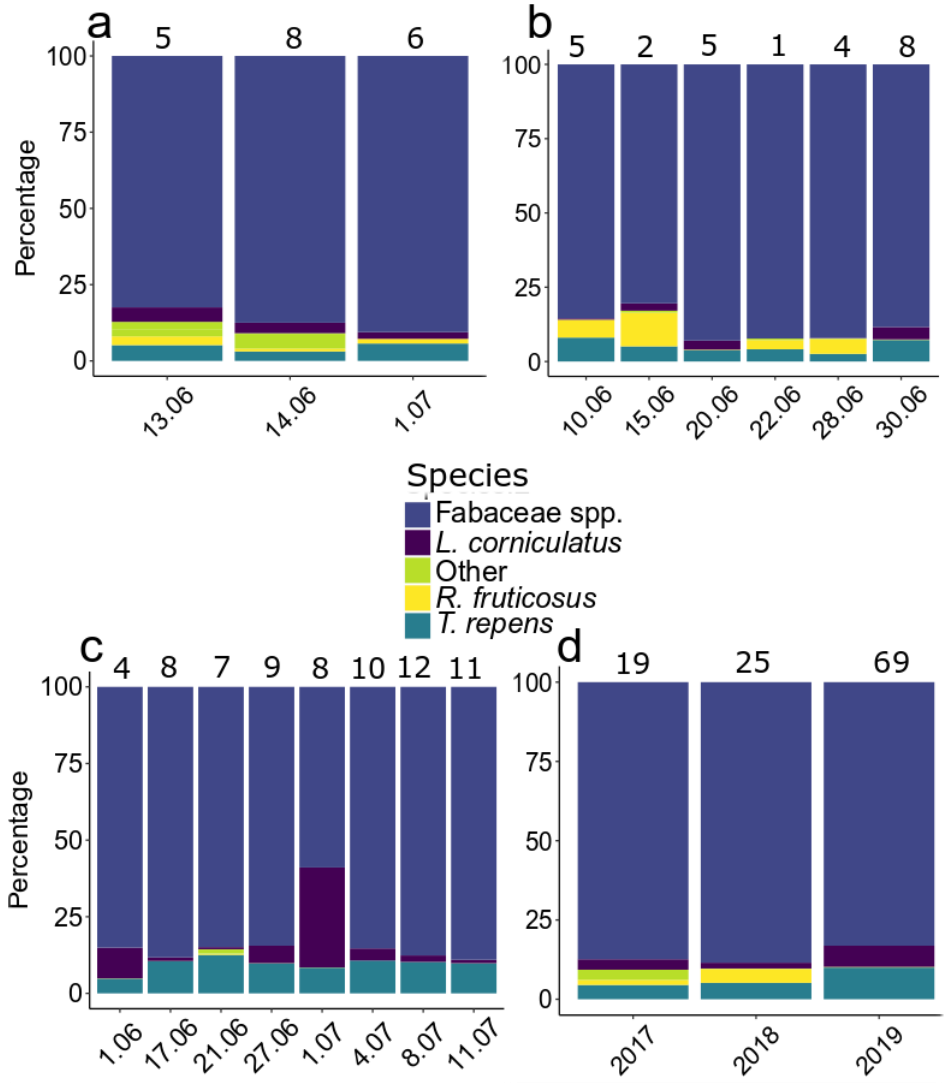
N	125.22	16.03	101.86	166.83	N	51.63	11.44	38.47	87.94

5- 2019 Aggregation 2

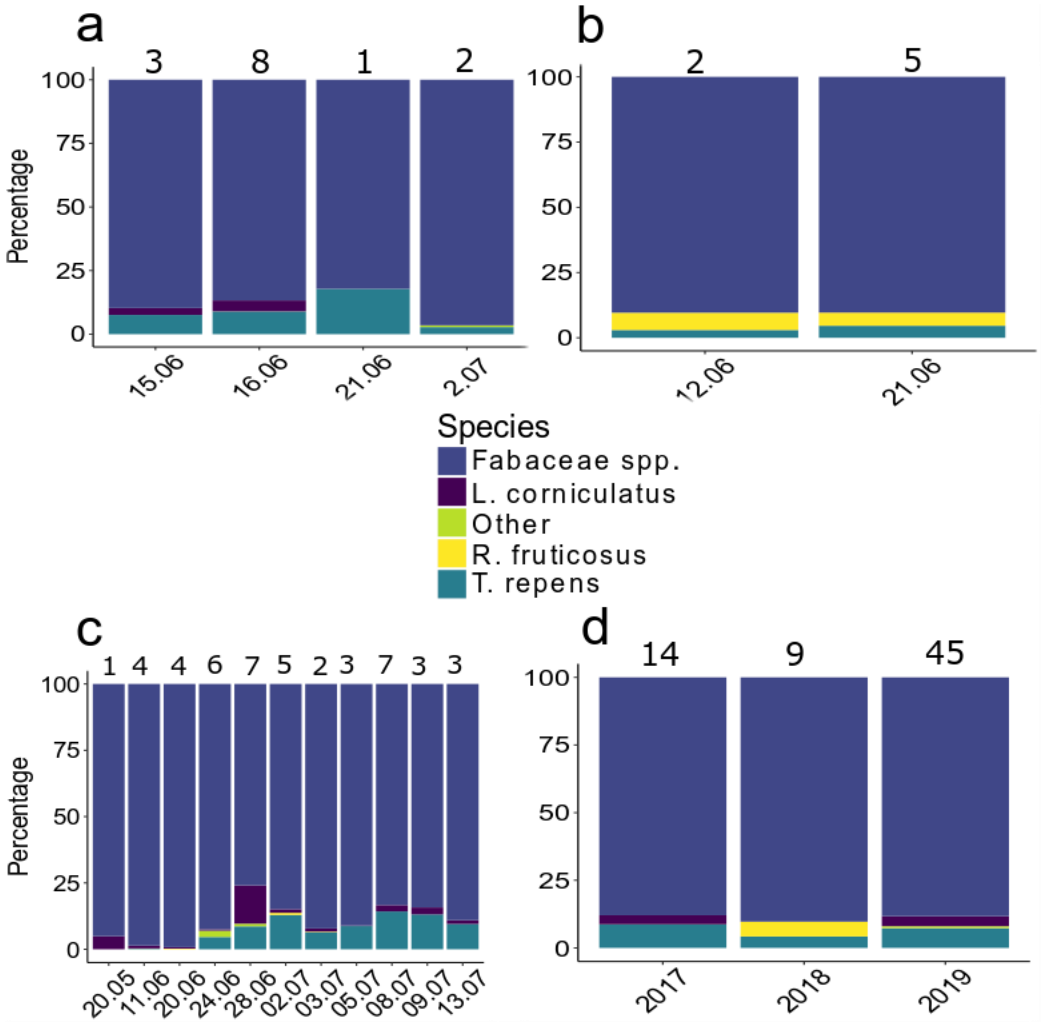
a) Females

			95% Confidence Interval		b) Males			95% Confidence Interval	
Parameter	Estimate	SE	Lower	Upper	Parameter	Estimate	SE	Lower	Upper
Phi1	0.93	0.01	0.90	0.95	Phi1	0.82	0.04	0.72	0.89
p1	0.25	0.05	0.16	0.37	p1	0.79	0.25	0.16	0.99
Pent1	0.43	0.11	0.23	0.65	Pent1	0.13	0.09	0.03	0.41
Pent2	0.06	0.11	0.00	0.68	Pent2	0.03	0.04	0.00	0.35
Pent3	0.10	0.09	0.02	0.43	Pent3	0.15	0.07	0.06	0.35
Pent4	0.07	0.07	0.01	0.40					
Pent5	0.10	0.08	0.02	0.38					
Pent6	0.10	0.07	0.02	0.34					
Pent7	0.03	0.04	0.00	0.36					
N	149.18	22.12	116.73	206.26	N	30.69	8.06	26.47	72.66

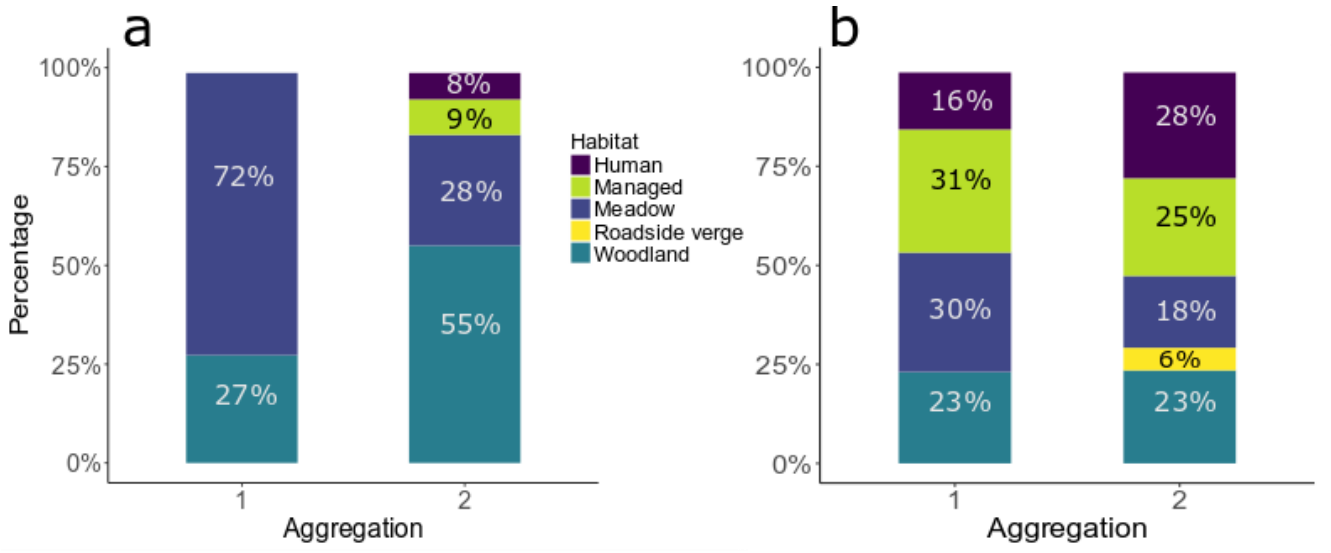
C5: Proportions of pollen grains for the most common plant species per day for aggregation 1. a; 2017, b; 2018, c; 2019 and d; the proportion of each species in pollen samples for the year. Other comprises plant species which overall accounted for less than 5% of samples. Numbers above the bars indicate number of bees sampled. Fabaceae spp. does not include *L. corniculatus* and *T. repens*



C6: Proportions of pollen grains for the most common plant species per day for aggregation 2. a; 2017, b; 2018, c; 2019 and d; the proportion of each species in pollen samples for the year. Other comprises plant species which overall accounted for less than 5% of samples. Numbers above the bars indicate number of bees sampled. Fabaceae spp does not include *L. corniculatus* and *T. repens*



C7: The composition of the surrounding landscape of the two aggregations, a; within 100m, b; within 500m. For definition of habitat types see methods.



Appendix D: Chapter Seven

D1: Final model summary outputs of both linear models and general linear models for both *Apis mellifera* and *Bombus terrestris/lucorum*. The effects of wind speed, bee species, temperature and the interactions between temperature and bee species, wind speed and bee species and wind speed and temperature on (a) number of flower visits (log), (b) mean handling time (square root transformed), (c) mean duration of inter flower flight and (d) proportion of flowers walked to. Bold indicates a significant effect on the response variable.

		Bombus terrestris/lucorum				***Apis mellifera***			
	Fixed effects	Estimate	SE	t	P	Estimate	SE	t	P
(a) Flower visits	Intercept	1.51				1.22			
	Wind speed	-0.03	0.01	-1.79	0.077	**-0.06**	**0.01**	**-8.78**	**<0.001**
	Bee species	**-0.29**	**5.39**	**-19.56**	**<0.001**	**0.29**	**0.01**	**19.65**	**<0.001**
	Temperature	**-0.02**	**0.00**	**6.05**	**<0.001**	-0.01	0.00	-1.95	0.052
	Wind speed *bee species	**0.04**	**0.02**	**-2.36**	**0.019**	**0.04**	**0.02**	**2.36**	**0.019**
	Temperature* Bee species	**-0.03**	**0.01**	**-5.85**	**<0.001**	**0.03**	**0.01**	**5.95**	**<0.001**
	Wind speed* temperature	0.00	0.00	1.30	0.20	0.00	0.00	1.30	0.20
(b) Mean handling time	Intercept	1.14				1.82			
	Wind speed	0.05	0.03	2.01	0.050	**0.13**	**0.01**	**9.82**	**<0.001**
	Bee species	**0.677**	**0.04**	**17.56**	**<0.001**	**-0.68**	**0.04**	**-17.46**	**<0.001**
	Temperature^2	**0.02**	**0.00**	**7.14**	**<0.001**	0.00	0.00	-1.12	0.266
	Average wind speed *bee species	**0.08**	**0.03**	**2.67**	**0.09**	**-0.08**	**0.03**	**-2.67**	**0.008**
	Temperature*bee species	**-0.02**	**0.00**	**-6.32**	**<0.001**	**0.02**	**0.00**	**6.32**	**<0.001**
	Wind speed* temperature	0.00	0.00	-1.61	0.108	0.00	0.00	-1.61	0.108
(c) Mean duration of inter flower flight	Intercept	-0.35				0.14			
	Average wind speed	0.00	0.12	0.04	0.966	0.00	0.02	0.19	0.848
	Bee species	**0.48**	**0.11**	**4.38**	**<0.001**	-0.56	0.03	-1.68	0.098
	Temperature	-0.05	0.03	-1.53	0.128	**-0.08**	**0.02**	**3.78**	**<0.001**
	Average wind speed *bee species	0.00	0.12	0.01	0.991	0.02	0.03	0.63	0.526
	Temperature*bee species	**0.12**	**0.04**	**3.38**	**<0.001**	**0.35**	**0.01**	**-2.55**	**0.012**
	Wind speed* temperature	-0.02	0.02	-1.32	0.189	-0.02	0.02	-1.32	0.189
(d) Proportion of flowers walked to	Intercept	-0.31				-0.48			
	Average wind speed	0.03	0.04	0.90	0.371	**-0.05**	**0.02**	**-2.64**	**0.009**
	Bee species	**-0.17**	**0.04**	**-4.38**	**<0.001**	**0.17**	**0.04**	**4.38**	**<0.001**
	Temperature	0.02	0.01	1.57	0.117	-0.02	0.01	-1.81	0.072
	Average wind speed *bee species	**-0.09**	**0.04**	**-2.01**	**0.048**	**0.09**	**0.04**	**2.01**	**0.048**
	Temperature*bee species	**-0.03**	**0.01**	**-2.36**	**0.019**	**0.03**	**0.01**	**2.36**	**0.019**
	Wind speed* temperature	0.00	0.01	0.26	0.799	0.00	0.01	0.26	0.799

Appendix E: Chapter Eight

E1: Pairwise comparisons conducted using the emmeans function from the emmeans package in R (Lenth et al 2019) of hesitancy durations for *Apis mellifera* and *Bombus* species when foraging on the seven studied plant species. Significant differences are indicated in bold.

Contrast	Ratio	SE	t ratio	*P* value
C. arvense Apis / C. scabiosa Apis	0.780	0.0625	-3.099	0.1068
C. arvense Apis / E. vulgare Apis	**0.614**	**0.0429**	**-6.981**	**<0.0001**
C. arvense Apis / J. vulgaris Apis	0.841	0.0846	-1.72	0.9089
C. arvense Apis / M. oficinalis Apis	0.947	0.0942	-0.548	1
C. arvense Apis / R. fruticosus Apis	**0.639**	**0.0546**	**-5.245**	**<0.0001**
C. arvense Apis / T. repens Apis	0.921	0.0799	-0.951	0.9996
C. arvense Apis / C. arvense Bombus	1.067	0.0802	0.865	0.9999
C. arvense Apis / C. scabiosa Bombus	0.868	0.0602	-2.048	0.7367
C. arvense Apis / E. vulgare Bombus	1.061	0.0726	0.87	0.9999
C. arvense Apis / J. vulgaris Bombus	0.913	0.0979	-0.845	0.9999
C. arvense Apis / M. oficinalis Bombus	0.916	0.1047	-0.77	1
C. arvense Apis / R. fruticosus Bombus	1.029	0.0769	0.388	1
C. arvense Apis / T. repens Bombus	1.233	0.1594	1.619	0.9414
C. scabiosa Apis / E. vulgare Apis	0.787	0.0635	-2.965	0.1521
C. scabiosa Apis / J. vulgaris Apis	1.078	0.0871	0.933	0.9997
C. scabiosa Apis / M. oficinalis Apis	1.214	0.1001	2.352	0.5159
C. scabiosa Apis / R. fruticosus Apis	0.819	0.0833	-1.967	0.7883
C. scabiosa Apis / T. repens Apis	1.18	0.1234	1.587	0.9496
C. scabiosa Apis / C. arvense Bombus	**1.368**	**0.1092**	**3.927**	**0.0068**
C. scabiosa Apis / C. scabiosa Bombus	1.112	0.0772	1.533	0.9616
C. scabiosa Apis / E. vulgare Bombus	**1.361**	**0.0978**	**4.284**	**0.0016**
C. scabiosa Apis / J. vulgaris Bombus	1.171	0.1208	1.53	0.9622
C. scabiosa Apis / M. oficinalis Bombus	1.174	0.1302	1.446	0.9763
C. scabiosa Apis / R. fruticosus Bombus	**1.32**	**0.1066**	**3.436**	**0.0392**
C. scabiosa Apis / T. repens Bombus	**1.58**	**0.2102**	**3.441**	**0.0385**
E. vulgare Apis / J. vulgaris Apis	1.369	0.141	3.054	0.1209
E. vulgare Apis / M. oficinalis Apis	**1.542**	**0.1549**	**4.308**	**0.0014**
E. vulgare Apis / R. fruticosus Apis	1.04	0.086	0.47	1
E. vulgare Apis / T. repens Apis	**1.499**	**0.1255**	**4.839**	**0.0001**
E. vulgare Apis / C. arvense Bombus	**1.738**	**0.1281**	**7.494**	**<0.0001**
E. vulgare Apis / C. scabiosa Bombus	**1.413**	**0.0959**	**5.089**	**<0.0001**
E. vulgare Apis / E. vulgare Bombus	**1.728**	**0.1111**	**8.509**	**<0.0001**
E. vulgare Apis / J. vulgaris Bombus	**1.487**	**0.1587**	**3.721**	**0.0148**
E. vulgare Apis / M. oficinalis Bombus	**1.491**	**0.169**	**3.523**	**0.0294**
E. vulgare Apis / R. fruticosus Bombus	**1.676**	**0.1223**	**7.076**	**<0.0001**
E. vulgare Apis / T. repens Bombus	**2.007**	**0.2608**	**5.363**	**<0.0001**
J. vulgaris Apis / M. oficinalis Apis	1.126	0.0946	1.411	0.9808

J. vulgaris Apis / R. fruticosus Apis	0.759	0.0948	-2.206	0.6252
J. vulgaris Apis / T. repens Apis	1.095	0.1409	0.704	1
J. vulgaris Apis / C. arvense Bombus	1.269	0.1252	2.412	0.4719
J. vulgaris Apis / C. scabiosa Bombus	1.031	0.0931	0.343	1
J. vulgaris Apis / E. vulgare Bombus	1.262	0.117	2.508	0.4027
J. vulgaris Apis / J. vulgaris Bombus	1.086	0.1183	0.757	1
J. vulgaris Apis / M. oficinalis Bombus	1.089	0.128	0.723	1
J. vulgaris Apis / R. fruticosus Bombus	1.224	0.1252	1.976	0.7827
J. vulgaris Apis / T. repens Bombus	1.466	0.2159	2.595	0.344
M. oficinalis Apis / R. fruticosus Apis	0.674	0.0818	-3.248	0.0699
M. oficinalis Apis / T. repens Apis	0.972	0.121	-0.225	1
M. oficinalis Apis / C. arvense Bombus	1.127	0.1106	1.218	0.995
M. oficinalis Apis / C. scabiosa Bombus	0.916	0.0817	-0.982	0.9995
M. oficinalis Apis / E. vulgare Bombus	1.121	0.1023	1.25	0.9936
M. oficinalis Apis / J. vulgaris Bombus	0.965	0.1066	-0.326	1
M. oficinalis Apis / M. oficinalis Bombus	0.967	0.1135	-0.285	1
M. oficinalis Apis / R. fruticosus Bombus	1.087	0.1088	0.835	0.9999
M. oficinalis Apis / T. repens Bombus	1.302	0.1895	1.812	0.8708
R. fruticosus Apis / T. repens Apis	**1.442**	**0.1267**	**4.167**	**0.0026**
R. fruticosus Apis / C. arvense Bombus	**1.671**	**0.1505**	**5.704**	**<0.0001**
R. fruticosus Apis / C. scabiosa Bombus	**1.359**	**0.1181**	**3.527**	**0.029**
R. fruticosus Apis / E. vulgare Bombus	**1.662**	**0.145**	**5.823**	**<0.0001**
R. fruticosus Apis / J. vulgaris Bombus	1.43	0.1761	2.908	0.1752
R. fruticosus Apis / M. oficinalis Bombus	1.434	0.1846	2.8	0.2252
R. fruticosus Apis / R. fruticosus Bombus	**1.612**	**0.1419**	**5.424**	**<0.0001**
R. fruticosus Apis / T. repens Bombus	**1.931**	**0.2573**	**4.935**	**0.0001**
T. repens Apis / C. arvense Bombus	1.159	0.1058	1.615	0.9423
T. repens Apis / C. scabiosa Bombus	0.942	0.084	-0.668	1
T. repens Apis / E. vulgare Bombus	1.153	0.1024	1.6	0.9464
T. repens Apis / J. vulgaris Bombus	0.992	0.1255	-0.063	1
T. repens Apis / M. oficinalis Bombus	0.994	0.131	-0.042	1
T. repens Apis / R. fruticosus Bombus	1.118	0.0994	1.254	0.9934
T. repens Apis / T. repens Bombus	1.339	0.1792	2.179	0.6447
C. arvense Bombus / C. scabiosa Bombus	0.813	0.0594	-2.835	0.2078
C. arvense Bombus / E. vulgare Bombus	0.995	0.0688	-0.079	1
C. arvense Bombus / J. vulgaris Bombus	0.856	0.0952	-1.399	0.9821
C. arvense Bombus / M. oficinalis Bombus	0.858	0.1015	-1.294	0.9911
C. arvense Bombus / R. fruticosus Bombus	0.965	0.0716	-0.485	1
C. arvense Bombus / T. repens Bombus	1.155	0.1481	1.125	0.9977
C. scabiosa Bombus / E. vulgare Bombus	1.223	0.0816	3.024	0.1308
C. scabiosa Bombus / J. vulgaris Bombus	1.053	0.1008	0.539	1
C. scabiosa Bombus / M. oficinalis Bombus	1.056	0.1093	0.522	1
C. scabiosa Bombus / R. fruticosus Bombus	1.187	0.0913	2.223	0.6127
C. scabiosa Bombus / T. repens Bombus	1.421	0.1875	2.662	0.302
E. vulgare Bombus / J. vulgaris Bombus	0.861	0.0938	-1.377	0.9844
E. vulgare Bombus / M. oficinalis Bombus	0.863	0.0998	-1.276	0.9922

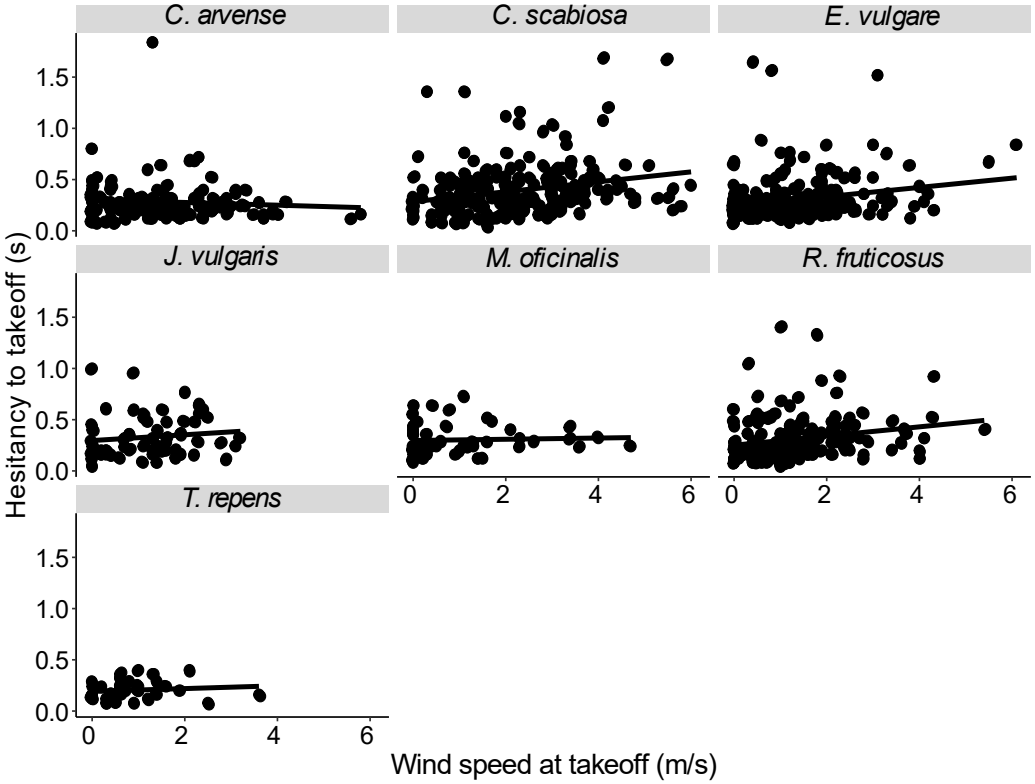
E. vulgare Bombus / R. fruticosus Bombus	0.97	0.0612	-0.484	1
E. vulgare Bombus / T. repens Bombus	1.162	0.1476	1.178	0.9964

E2: Final model summary outputs of the linear models for both *Apis mellifera* and *Bombus* species testing the effects of wind speed, plant species, temperature, bee group and flower movement and the interactions between wind speed * plant species, wind speed*bee group, plant species*bee group and bee group*flower movement on logged hesitancy duration. Bold indicates a significant effect on the response variable.

	Apis mellifera				***Bombus spp.***			
	Estimate	Std. Error	t	*P*	Estimate	SE	t	*P*
(Intercept)	-1.315	0.055	-24.053	<0.001	-1.380	0.057	-24.233	< 0.001
Average flower movement	0.004	0.009	0.416	0.677	-0.002	0.008	-0.219	0.827
Wind speed	0.064	0.0382	1.679	0.093	0.064	0.038	1.679	0.093
Temperature	**-0.018**	**0.005**	**-3.417**	**<0.001**	**-0.018**	**0.005**	**-3.417**	**0.001**
Bee group	-0.065	0.075	-0.865	0.387	0.065	0.075	0.865	0.387
Plant species *C. scabiosa*	**0.251**	**0.080**	**3.132**	**0.002**	**0.209**	**0.073**	**2.879**	**0.004**
Plant species *E. vulgare*	**0.489**	**0.070**	**6.974**	**<0.001**	0.007	0.069	0.103	0.918
Plant species *J. vulgaris*	0.177	0.101	1.753	0.080	0.160	0.111	1.434	0.152
Plant species *M. oficinalis*	0.060	0.099	0.606	0.544	0.158	0.118	1.343	0.179
Plant species *R. fruticosus*	**0.450**	**0.086**	**5.232**	**<0.001**	0.037	0.074	0.493	0.622
Plant species *T. repens*	0.086	0.087	0.986	0.324	-0.141	0.129	-1.098	0.272
Average flower movement * Wind speed	**-0.009**	**0.004**	**-2.124**	**0.034**	**-0.009**	**0.004**	**-2.124**	**0.034**
Average flower movement* Temperature	-0.001	0.001	-1.341	0.180	-0.001	0.001	-1.341	0.180
Average flower movement * Bee group	-0.005	0.009	-0.568	0.570	0.005	0.009	0.568	0.570
Bee group*Plant.Species *C. scabiosa*	-0.041	0.105	-0.393	0.694	0.041	0.105	0.393	0.694
Bee group*Plant.Species *E. vulgare*	**-0.482**	**0.097**	**-4.992**	**<0.001**	**0.482**	**0.097**	**4.992**	**<0.001**
Bee group*Plant.Species *J. vulgaris*	-0.018	0.137	-0.128	0.899	0.018	0.137	0.128	0.898
Bee group*Plant.Species *M. oficinalis*	0.099	0.143	0.686	0.493	-0.099	0.144	-0.686	0.493
Bee group*Plant.Species *R. fruticosus*	**-0.413**	**0.112**	**-3.685**	**<0.001**	**0.413**	**0.112**	**3.685**	**<0.001**
Bee group*Plant.Species *T. repens*	-0.227	0.150	-1.512	0.131	0.227	0.150	1.512	0.131

Wind speed*Plant Species *C. scabiosa*	0.061	0.045	1.353	0.176	0.061	0.045	1.353	0.176
Wind speed*Plant Species *E. vulgare*	0.048	0.046	1.039	0.230	0.048	0.046	1.039	0.299
Wind speed*Plant Species *J. vulgaris*	0.115	0.065	1.772	0.077	0.115	0.065	1.772	0.077
Wind speed*Plant Species *M. oficinalis*	**0.152**	**0.067**	**2.279**	**0.023**	**0.152**	**0.067**	**2.279**	**0.023**
Wind speed*Plant Species *R. fruticosus*	0.019	0.055	0.343	0.732	0.019	0.055	0.343	0.732
Wind speed*Plant Species *T. repens*	0.085	0.062	1.372	0.170	0.085	0.062	1.372	0.170

E3: *Bombus* species hesitancy to take off (s) in relation to wind speed (ms^{-1}) separated by plant species being foraged. Regression lines are shown, and each point is an individual bee. *N=898*



E4: *Apis mellifera* hesitancy to take off (s) in relation to wind speed (ms^{-1}) separated by plant species being foraged. Regression lines are shown, and each point is an individual bee. *N=827*